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<p>(54) Title: PHARMACEUTICAL COMPOSITIONS FOR THE NASAL DELIVERY OF COMPOUNDS USEFUL FOR THE TREATMENT OF OSTEOPOROSIS</p>		
<p>(57) Abstract</p> <p>A pharmaceutical composition for the nasal delivery of compounds useful for treating osteoporosis, comprising an effective amount of a physiologically active truncated analog of PTH or PTHrp, or salt thereof, in which amino acid residues (22-31) form an amphipathic <math>\alpha</math>-helix, said residues (22-31) selected from (SEQ ID NOS: 85, 86, 26, 27, 28, 29, and 30); an absorption enhancer selected from the group consisting of dimethyl-<math>\beta</math>-cyclodextrin and the bile acid surfactants; and water is provided.</p>		

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PHARMACEUTICAL COMPOSITIONS FOR THE NASAL DELIVERY OF  
COMPOUNDS USEFUL FOR THE TREATMENT OF OSTEOPOROSIS

BACKGROUND OF THE INVENTION

a) Field of the Invention

This invention relates to pharmaceutical compositions for the treatment of osteoporosis via the nasal administration of therapeutically effective amounts of certain novel analogs of parathyroid hormone and parathyroid hormone related peptide.

b) Description of Related Art

Osteoporosis is the most common form of metabolic bone disease and may be considered the symptomatic, fracture stage of bone loss (osteopenia). Although osteoporosis may occur secondary to a number of underlying diseases, 90% of all cases appear to be idiopathic. Postmenopausal women are particularly at risk for idiopathic osteoporosis (postmenopausal or Type I osteoporosis). Another high risk group for idiopathic osteoporosis is the elderly of either sex (senile or Type II osteoporosis). Osteoporosis has also been related to corticosteroid use, immobilization or extended bed rest, alcoholism, diabetes, gonadotoxic chemotherapy, hyperprolactinemia, anorexia nervosa, primary and secondary amenorrhea, and oophorectomy.

In the various forms of osteoporosis, bone fractures, which are the result of bone loss that has reached the point of mechanical failure, frequently occur. Postmenopausal osteoporosis is characterized by fractures of the wrist and spine, while femoral neck fractures seem to be the dominant feature of senile osteoporosis.

The mechanism by which bone is lost in osteoporotics is believed to involve an imbalance in the process by which the skeleton renews itself. This process has been termed bone remodeling. It occurs in a series of discrete pockets of activity. These pockets appear spontaneously within the bone matrix on a given bone surface as a site of bone resorption. Osteoclasts (bone dissolving or resorbing cells) are responsible for the resorption of a portion of bone of generally constant dimension. This resorption process is

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followed by the appearance of osteoblasts (bone forming cells) which then refill with new bone the cavity left by the osteoclasts.

5 In a healthy adult subject, the rate at which osteoclasts and osteoblasts are formed is such that bone formation and bone resorption are in balance. However, in osteoporotics an imbalance in the bone remodeling process develops which results in bone being lost at a rate faster than it is being made. Although this imbalance occurs to some extent in most  
10 individuals as they age, it is much more severe and occurs at a younger age in postmenopausal osteoporotics or following oophorectomy.

Adachi, et al. in *Seminars in Arthritis and Rheumatism*, 22:6, 375-84 (June 1993) report that despite much conflicting  
15 data regarding the pathophysiology of corticosteroid induced osteoporosis, it is generally agreed that there is a relative decrease in bone formation and a relative increase in bone resorption. Bone loss with resulting fractures and osteonecrosis is a frequent consequence of corticosteroid  
20 therapy. There is evidence that bone loss occurs rapidly within the first 6 to 12 months of corticosteroid therapy; there also appears to be a close relationship between rate of bone loss and corticosteroid dose. Men are equally susceptible to the effects of corticosteroids. The estimated  
25 incidence of fractures and osteonecrosis ranges from 30 to 50%.

There have been many attempts to treat osteoporosis with the goal of either slowing further bone loss or, more desirably, producing a net gain in bone mass. Certain agents,  
30 such as estrogen and the bisphosphonates, appear to slow further bone loss in osteoporotics. Agents which slow bone loss, because of the different durations of bone resorption and formation, may appear to increase bone mass (on the order of 3 to 7%). However, this apparent increase is limited in  
35 time, not progressive, and is due to a decrease in "remodeling space." In addition, because of the close coupling between resorption and formation, treatments which impede bone

resorption also ultimately impede bone formation.

It has been suggested that treatment with parathyroid hormone (PTH) would lead to both increased bone turnover and a positive calcium balance. However, human clinical trials have shown that any increase in trabecular bone is offset by a decrease in cortical bone, so that there is no net increase in total bone.

Hefti, et al. in *Clinical Science* 62, 389-396 (1982) have reported that daily subcutaneous doses of either bPTH(1-84) or hPTH(1-34) increased whole body calcium and ash weight of individual bones in both normal and osteoporotic adult female rats.

Liu, et al. in *J. Bone Miner. Res.* 6:10, 1071-1080 (1991) have noted that ovariectomy of adult female rats induced a 47% loss in the percentage of trabecular bone in the proximal tibial metaphysis, accompanied by a significant increase in the number of osteoblasts and trabecular osteoclasts. Daily subcutaneous injections of hPTH(1-34) completely reversed the loss of trabecular bone and resulted in amounts of trabecular bone exceeding that of sham operated controls. The number of osteoblasts increased and the number of osteoclasts decreased.

Hock et al. in *J. Bone Min. Res.* 7:1, 65-71 (1992) have reported that daily subcutaneous injections of hPTH(1-34) to healthy adult male rats for 12 days increased trabecular and cortical bone calcium and dry weight. Total bone mass, trabecular bone volume, trabecular thickness and number, and osteoblastic surfaces were increased.

The mammalian parathyroid hormones, e.g. human (hPTH), bovine (bPTH), and porcine (pPTH), are single polypeptide chains of 84 amino acid residues, with molecular weights of approximately 9500. Biological activity is associated with the N-terminal portion, with residues (1-34) apparently the minimum required.

The N-terminal segment of human PTH differs from the N-terminal segment of the bovine and porcine hormones by only three and two amino acid residues, respectively:

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hPTH(1-34):

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu  
 1 5 10 15  
 5 Asn Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp  
 20 25 30

Val His Asn Phe (SEQ ID NO:1);

bPTH(1-34):

Ala Val Ser Glu Ile Gln Phe Met His Asn Leu Gly Lys His Leu  
 1 5 10 15  
 15 Ser Ser Met Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp  
 20 25 30

Val His Asn Phe (SEQ ID NO:2);

pPTH(1-34):

Ser Val Ser Glu Ile Gln Leu Met His Asn Leu Gly Lys His Leu  
 1 5 10 15  
 25 Ser Ser Leu Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp  
 20 25 30

Val His Asn Phe (SEQ ID NO:3).

The primary function of PTH is to elicit the adaptive changes that serve to maintain a constant concentration of  $\text{Ca}^{2+}$  in the extracellular fluid. PTH acts on the kidneys to increase tubular reabsorption of  $\text{Ca}^{2+}$  from the urine, as well as stimulating the conversion of calcifediol to calcitriol, which is responsible for absorption of  $\text{Ca}^{2+}$  from the intestines. One prominent effect is to promote the mobilization of  $\text{Ca}^{2+}$  from bone. PTH acts on bone to increase the rate of resorption of  $\text{Ca}^{2+}$  and phosphate. PTH stimulates the rate of bone resorption by osteoclasts, increases the rate of differentiation of mesenchymal cells to osteoclasts, and prolongs the half life of these latter cells. With prolonged action of PTH the number of bone forming osteoblasts is also increased; thus, the rate of bone turnover and remodeling is enhanced. However, individual osteoblasts appear to be less

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active than normal.

Rosenblatt, et al. in U.S. Patent Nos. 4,423,037, 4,968,669 and 5,001,223 have disclosed PTH antagonists obtained by the deletion of the N-terminal (1-6) amino acids and the selective replacement of Phe<sup>7</sup>, Met<sup>8,18</sup>, and Gly<sup>12</sup>. Tyr<sup>34</sup>-NH<sub>2</sub> reportedly increased the activity and stability of these compounds.

Parathyroid hormone-related peptide (PTHrp), a 140+ amino acid protein, and fragments thereof, reproduce the major biological actions of PTH. PTHrp is elaborated by a number of human and animal tumors and other tissues and may play a role in hypercalcemia of malignancy. The sequence of hPTHrp (1-34) is as follows:

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1				5				10					15	
Gln	Asp	Leu	Arg	Arg	Arg	Phe	Phe	Leu	His	His	Leu	Ile	Ala	Glu
			20					25					30	

Ile His Thr Ala (SEQ ID NO:4).

The sequence homology between hPTH and hPTHrp is largely limited to the 13 N-terminal residues, 8 of which are identical; only 1 of 10 amino acids in the (25-34) receptor binding region of hPTH is conserved in hPTHrp. Conformational similarity may underlie the common activity. Cohen, et al. in *J. Biol. Chem.* 266:3, 1997-2004 (1991) have suggested that much of the sequence of PTH(1-34) and PTHrp(1-34), in particular regions (5-18) and (21-34), assumes an  $\alpha$ -helical configuration, while noting that there is some question whether this configuration prevails for the carboxyl terminal end under physiological conditions. Such a secondary structure may be important for lipid interaction, receptor interaction, and/or structural stabilization.

We have synthesized analogs of PTH and of PTHrp with the objective of developing improved therapeutic agents for the restoration of bone mass in mammalian subjects, including those afflicted with osteoporosis. Delivery of these agents in the most efficacious manner possible is highly desirable,

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both in view of convenience to the patient and the expected high cost of the peptide. It seems likely that the compounds, analogous to PTH, will be most effectively delivered in a pulsatile fashion. While subcutaneous injection is compatible with this objective, there are decided disadvantages to this mode of delivery, not the least of which is the pain associated with daily injections. Oral delivery has unfortunately not been particularly useful for the delivery of peptide drugs, largely due to degradation in the gastrointestinal tract and resultant low (<1%) bioavailability of the ingested drug.

Alternative, non-traditional, modes of delivery, such as transdermal, pulmonary, and nasal may prove more attractive for the delivery of these labile agents. The current invention encompasses the discovery and development of liquid compositions with particular utility for nasal delivery. Nasal delivery of peptides has found some limited commercial success, such as with Synarel® Nasal Solution (Syntex Corporation, Palo Alto, CA), a potent decapeptide (nafarelin) indicated for the treatment of endometriosis, for which bioavailability up to about 3% has been obtained. Enhanced nasal formulations of LH-RH analogs, including nafarelin, with significantly higher bioavailabilities, have been reported by Anik in U.S. Patent Nos. 4,476,116 and 5,116,817. Merkus et al. have reported in PCT Patent Publication No. 92/01440 compositions for the nasal administration of peptides, particularly insulin, including dimethyl- $\beta$ -cyclodextrin as an absorption enhancer. Aliverti et al. in U.S. Patent No. 5,183,802 disclose a pharmaceutical composition of calcitonin and a glycyrrhizinate absorption enhancer for nasal administration in the treatment of osteoporosis. However, none of the nasal compositions developed to date is entirely satisfactory at delivering peptide compounds with acceptable levels of bioavailability.

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SUMMARY OF THE INVENTION

This invention provides a pharmaceutical composition for the nasal delivery of compounds useful for treating osteoporosis, comprising an effective amount of a physiologically active truncated analog of PTH or PTHrp, or salt thereof, in which amino acid residues (22-31) form an amphipathic  $\alpha$ -helix, said residues (22-31) selected from (SEQ ID NOS: 85, 86, 26, 27, 28, 29, and 30); an absorption enhancer selected from the group consisting of dimethyl- $\beta$ -cyclodextrin and the bile acid surfactants; and water.

DETAILED DESCRIPTION OF THE INVENTIONAbbreviations and Definitions

The one- and three-letter abbreviations for the various common nucleotide bases and amino acids are as recommended in *Pure Appl. Chem.* 31, 639-645 (1972) and 40, 277-290 (1974) and the IUPAC-IUB Biochemical Nomenclature Commission and comply with 37 CFR §1.822 (55 FR 18245, May 1, 1990). The one- and three-letter abbreviations are as follows:

Amino Acid Abbreviations

Amino Acid	Three-letter Symbol	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic Acid	Asp	D
Asn + Asp	Asx	B
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Gln + Glu	Glx	Z
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L



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	Lysine	Lys	K
	Methionine	Met	M
	Phenylalanine	Phe	F
	Proline	Pro	P
5	Serine	Ser	S
	Threonine	Thr	T
	Tryptophan	Trp	W
	Tyrosine	Tyr	Y
	Valine	Val	V
10	Other amino acid	Xaa	X

The abbreviations represent L-amino acids unless otherwise designated as D- or D,L-. Certain amino acids, both natural and non-natural, are achiral, e.g. glycine. All peptide sequences are presented with the N-terminal amino acid on the left and the C-terminal amino acid on the right.

Further abbreviations for other amino acids and compounds used herein are:

	hSer	homoserine
	hSerlac	homoserine lactone
20	Nle	norleucine
	PEG2	radical of diethylene glycol methyl ether, a.k.a. methoxydi(ethyleneoxy), $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_2-$ , (MW = 119)
25	PEG5000	radical of poly(ethylene glycol methyl ether), a.k.a. methoxypoly(ethyleneoxy), $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_{110}-$ , (avg. MW = 5000)
30	PEGX	radical of poly(ethylene glycol methyl ether), $\text{CH}_3\text{O}(\text{CH}_2\text{CH}_2\text{O})_n-$ , $n = 2 - 225$ , (avg. MW = 100 to 10,000)

"Hydrophilic amino acid (Haa)" refers to an amino acid having at least one hydrophilic functional group in addition to those required for peptide bond formation, such as arginine, asparagine, aspartic acid, glutamic acid, glutamine, histidine, lysine, serine, threonine, and their homologs.

"Lipophilic amino acid (Laa)" refers to an uncharged, aliphatic or aromatic amino acid, such as isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, valine, and

their homologs.

For the purposes of this invention, alanine is classified as "amphiphilic" i.e., capable of acting as either hydrophilic or lipophilic.

5 "Physiologically active truncated homolog or analog of PTH or PTHrp" refers to a polypeptide having a sequence comprising less than the full complement of amino acids found in PTH or PTHrp which, however, elicits a similar physiological response. The truncated PTH or PTHrp need not  
10 be fully homologous with PTH or PTHrp to elicit a similar physiological response. PTH(1-34) and PTHrp(1-34) are preferred, but not exclusive, representatives of this group.

"Amphipathic  $\alpha$ -helix" refers to the secondary structure exhibited by certain polypeptides in which the amino acids  
15 assume an  $\alpha$ -helical configuration having opposing polar and nonpolar faces oriented along the long axis of the helix. The possibility of  $\alpha$ -helical structure in the polypeptide of interest may be explored to some extent by the construction of a "Schiffer-Edmundson wheel" (M. Schiffer and A. B. Edmundson,  
20 *Biophys. J.* 7, 121 (1967)), of the appropriate pitch and noting the segregation of the hydrophilic and lipophilic residues on opposite faces of the cylinder circumscribing the helix. Alternatively, empirical evidence, such as circular  
25 dichroism or x-ray diffraction data, may be available indicating the presence of an  $\alpha$ -helical region in a given polypeptide. An ideal  $\alpha$ -helix has 3.6 amino acid residues per turn with adjacent side chains separated by 100° of arc. Eisenberg et al. in *Nature* 299:371-374 (1982) and *Proc. Nat. Acad. Sci. USA* 81:140-144 (1984) have combined a  
30 hydrophobicity scale with the helical wheel to quantify the concept of amphipathic helices. The mean hydrophobic moment is defined as the vector sum of the hydrophobicities of the component amino acids making up the helix. The following hydrophobicities for the amino acids are those reported by  
35 Eisenberg (1984) as the "consensus" scale:

Ile 0.73; Phe 0.61; Val 0.54; Leu 0.53; Trp 0.37;  
Met 0.26 Ala 0.25; Gly 0.16; Cys 0.04; Tyr 0.02;

Pro -0.07; Thr -0.18; Ser -0.26; His -0.40; Glu -0.62;  
Asn -0.64; Gln -0.69; Asp -0.72; Lys -1.10; Arg -1.76.

The hydrophobic moment,  $\mu_H$ , for an ideal  $\alpha$ -helix having  
3.6 residues per turn (or a  $100^\circ$  arc ( =  $360^\circ/3.6$ ) between  
side chains), may be calculated from:

$$\mu_H = [ ( \sum H_N \sin \delta (N-1) )^2 + ( \sum H_N \cos \delta (N-1) )^2 ]^{1/2},$$

where  $H_N$  is the hydrophobicity value of the  $N^{\text{th}}$  amino acid and  
the sums are taken over the  $N$  amino acids in the sequence with  
periodicity  $\delta=100^\circ$ . The hydrophobic moment may be expressed  
as the mean hydrophobic moment per residue by dividing  $\mu_H$  by  $N$   
to obtain  $\langle \mu_H \rangle$ . A value of  $\langle \mu_H \rangle$  at  $100^\circ \pm 20^\circ$  of about 0.20 or  
greater is suggestive of amphipathic helix formation. The  
 $\langle \mu_H \rangle$  values at  $100^\circ$  for hPTHrp (22-31) and hPTH (22-31) are  
0.19 and 0.37, respectively.

Cornett, et al., in *J. Mol. Biol.*, 195:659-685 (1987)  
have further extended the study of amphipathic  $\alpha$ -helices by  
introducing the "amphipathic index" as a predictor of  
amphipathicity. They concluded that approximately half of all  
known  $\alpha$ -helices are amphipathic, and that the dominant  
frequency is  $97.5^\circ$  rather than  $100^\circ$ , with the number of  
residues per turn being closer to 3.7 than 3.6. While such  
refinements are scientifically interesting, the basic approach  
of Eisenberg, et al. is sufficient to classify a given  
sequence as amphipathic, particularly when one is designing a  
sequence *ab initio* to form an amphipathic  $\alpha$ -helix.

A substitute amphipathic  $\alpha$ -helical amino acid sequence  
may lack homology with the sequence of a given segment of a  
naturally occurring polypeptide but elicits a similar  
secondary structure, i. e. an  $\alpha$ -helix having opposing polar  
and nonpolar faces, in the physiological environment.  
Replacement of the naturally occurring amino acid sequence  
with an alternative sequence may beneficially affect the  
physiological activity, stability, or other properties of the  
altered parent polypeptide. Guidance as to the design and  
selection of such sequences is provided in J. L. Krstenansky,  
et al., *FEBS Letters* 242:2, 409-413 (1989), and J. P. Segrest,  
et al. *Proteins: Structure, Function, and Genetics* 8:103-117

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(1990) among others.

The ten amino acid amphipathic  $\alpha$ -helix of this invention has the formula:

Haa (Laa Laa Haa Haa)<sub>2</sub> Laa

5 wherein the Haa's are selected from the group of hydrophilic amino acids and the Laa's are selected from the group of lipophilic amino acids, as defined above. Assuming an idealized  $\alpha$ -helix, residues 1, 4, 5, 8, and 9 are distributed along one face (A) of the helix within about a 140° arc of each other, while residues 2, 3, 6, 7, and 10 occupy an opposing 140° arc on the other face (B) of the helix. Preferably, all the residues on one face are of the same polarity while all those on the other face are of the opposite polarity, i.e., if face A is all hydrophilic, face B is all lipophilic and vice versa. The skilled artisan will recognize that while the helices of this invention are described by

Haa(Laa Laa Haa Haa)<sub>2</sub> Laa,

the reverse sequence,

20 Laa (Haa Haa Laa Laa)<sub>2</sub> Haa

will also meet the residue distribution criteria and is an equivalent descriptor of the helices of this invention.

25 Alanine may be substituted for either hydrophilic or lipophilic amino acids, since Ala can reside readily on either face of an amphipathic  $\alpha$ -helix, although Ala<sub>10</sub> does not form an amphipathic  $\alpha$ -helix. Generally, proline, cysteine, and tyrosine are not used; however, their presence and other random errors in the sequence may be tolerated, e.g. a hydrophilic residue on the lipophilic face, as long as the remaining amino acids in the segment substantially conform to the hydrophilic face - lipophilic face division. A convenient method for determining if a sequence is sufficiently amphipathic to be a sequence of this invention is to calculate the mean hydrophobic moment, as defined above. 35 If the peak mean moment per residue at 100°  $\pm$  20° exceeds about 0.20, then the sequence will form an amphipathic helix

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and is a sequence of this invention.

For example, the mean hydrophobic moment per residue at 100° for (SEQ ID NO: 26), Xaa = Glu, is calculated as follows:

	A.A.	H <sub>N</sub>	δ (N-1)	H sin δ(N-1)	H cos δ(N-1)
5	E	-.62	0	0	-.62
	L	.53	100	.52	-.17
	L	.53	200	-.18	-.50
	E	-.62	300	.34	-.31
10	K	-1.1	400	-.70	-.85
	L	.53	500	.34	-.41
	L	.53	600	-.46	-.27
	E	-.62	700	.21	-.58
	K	-1.1	800	-1.08	-.19
15	L	.53	900	0	-.53
				Σ=0.81	Σ=-4.43

$$\mu_H = [(0.81)^2 + (-4.43)^2]^{1/2} = 4.50$$

$$< \mu_H > = 4.50/10 = 0.45$$

For this sequence, the mean peak hydrophobic moment occurs at 92° and has a value of 0.48.

In applying this concept to parathyroid hormone and parathyroid hormone related peptide, it was hypothesized that either or both regions (7-16) and (22-31) may exhibit α-helical secondary structure and could be replaced with a non-homologous sequence having similar structural tendencies, without loss of biological activity or induction of immunoreaction.

#### Preferred Embodiments

In one aspect, this invention provides analogs of PTH, PTHrp, and the physiologically active truncated analogs and homologs of PTH and PTHrp, or salts thereof, in which amino acid residues (22-31) form an amphipathic α-helix, the

sequence of said residues (22-31) selected from:

a) Xaa<sup>1</sup> Xaa<sup>2</sup> Leu Xaa<sup>4</sup> Xaa<sup>5</sup> Leu Xaa<sup>7</sup> Xaa<sup>8</sup> Xaa<sup>9</sup> Xaa<sup>10</sup> wherein  
1 5 10

Xaa<sup>1</sup> and Xaa<sup>4</sup> are independently Glu, Glu(OCH<sub>3</sub>), His, or Phe; Xaa<sup>2</sup> is Leu or Phe; Xaa<sup>5</sup> is Lys or His; Xaa<sup>7</sup> and Xaa<sup>10</sup> are independently Leu or Ile; Xaa<sup>8</sup> is Ala, Arg, or Glu; and Xaa<sup>9</sup> is Lys or Glu (SEQ ID NO: 85); preferably

Glu Leu Leu Glu Lys Leu Leu Xaa Lys Leu wherein  
1 5 10

Xaa is Glu or Arg (SEQ ID NO:26);

b) Xaa<sup>1</sup> Xaa<sup>2</sup> Leu Xaa<sup>4</sup> Arg Leu Leu Xaa<sup>8</sup> Arg Leu wherein  
1 5 10

Xaa<sup>1</sup> and Xaa<sup>4</sup> are independently Glu, Glu(OCH<sub>3</sub>), His, or Phe; Xaa<sup>2</sup> is Leu or Phe; Xaa<sup>8</sup> is Glu, Lys, or Lys(COCH<sub>2</sub>PEGX) and PEGX is a poly-(ethylene glycol methyl ether) radical of molecular weight 100 to 10,000 (SEQ ID NO:86); preferably, Glu Leu Leu

Glu Arg Leu Leu Xaa Arg Leu wherein  
1 5

Xaa is Glu, Lys, or Lys(COCH<sub>2</sub>PEGX) and PEGX is a poly-(ethylene glycol methyl ether) radical of molecular weight 100 to 10,000 (SEQ ID NO:27);

c) Ala Leu Ala Glu Ala Leu Ala Glu Ala Leu (SEQ ID NO:28);  
1 5 10

d) Ser Leu Leu Ser Ser Leu Leu Ser Ser Leu (SEQ ID NO:29);  
1 5 10

e) Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu (SEQ ID NO:30).  
1 5 10

In another aspect, this invention provides analogs of PTH, PTHrp, and the physiologically active truncated analogs and homologs of PTH and PTHrp, or salts thereof, of the formula:

Xaa<sup>1</sup> Xaa<sup>2</sup> Xaa<sup>3</sup> Xaa<sup>4</sup> Xaa<sup>5</sup> Xaa<sup>6</sup> Xaa<sup>7</sup> Leu His Xaa<sup>10</sup> Xaa<sup>11</sup> Gly Xaa<sup>13</sup> Ser  
Ile Gln Xaa<sup>17</sup> Leu Xaa<sup>19</sup> Xaa<sup>20</sup> Xaa<sup>21</sup> Xaa<sup>22-31</sup> Xaa<sup>32</sup> Xaa<sup>33</sup> Xaa<sup>34</sup> Xaa<sup>35</sup> Xaa<sup>36</sup>  
Xaa<sup>37</sup> Xaa<sup>38</sup> Term, wherein:

Xaa<sup>1</sup> is absent or is Ala;

Xaa<sup>2</sup> is absent or is Val;

Xaa<sup>3</sup> is absent or is Ser;

Xaa<sup>4</sup> is absent or is Glu or Glu(OCH<sub>3</sub>);

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Xaa<sup>5</sup> is absent or is His or Ala;

Xaa<sup>6</sup> is absent or is Gln;

Xaa<sup>7</sup> is absent or is Leu;

Xaa<sup>10</sup> and Xaa<sup>17</sup> are independently Asp or Asp(OCH<sub>3</sub>);

5 Xaa<sup>11</sup> is Lys, Arg, or Leu;

Xaa<sup>13</sup> is Lys, Arg, Tyr, Cys, Leu, Cys(CH<sub>2</sub>CONH(CH<sub>2</sub>)<sub>2</sub>NH(biotinyl)),  
Lys(7-dimethylamino-2-oxo-2H-1-benzopyran-4-acetyl), or  
Lys(dihydrocinnamoyl);

Xaa<sup>20</sup> is Arg or Leu;

10 Xaa<sup>19</sup> and Xaa<sup>21</sup> are independently Lys, Ala, or Arg;

Xaa<sup>22-31</sup> is selected from (SEQ ID NOS:26, 27, 28, 29, or 30);

Xaa<sup>32</sup> is His, Pro, or Lys;

Xaa<sup>33</sup> is absent, or is Pro, Thr, Glu, or Ala;

Xaa<sup>34</sup> is absent, or is Pro, Arg, Met, Ala, hSer, hSer lactone,

15 Tyr, Leu, or 1,4-diaminobutyryl lactam;

Xaa<sup>35</sup> is absent or is Pro, Glu, Ser, Ala, or Gly;

Xaa<sup>36</sup> is absent or is Ala, Arg, or Ile;

Xaa<sup>37</sup> is absent or is Arg, Trp, or 3-(-2-naphthyl)-L-alanine;

Xaa<sup>38</sup> is absent or is Ala or hSer or Xaa<sup>38-42</sup> is Thr Arg Ser Ala

20 Trp;

and Term is OR or NR<sub>2</sub> where each R is independently H, (C<sub>1</sub>-C<sub>4</sub>)alkyl or phenyl(C<sub>1</sub>-C<sub>4</sub>)alkyl; and the pharmaceutically acceptable salts thereof.

In yet another aspect this invention includes polypeptide  
25 analogs of the physiologically active truncated homolog  
hPTHrp(1-34), as shown in Formula (I):

Ala Val Ser Glu Xaa<sup>5</sup> Gln Leu Leu His Asp Xaa<sup>11</sup> Gly Xaa<sup>13</sup> Ser Ile  
Gln Asp Leu Xaa<sup>19</sup> Arg Xaa<sup>21</sup> Xaa<sup>22-31</sup> Xaa<sup>32</sup> Xaa<sup>33</sup> Xaa<sup>34</sup> Term, wherein:

Xaa<sup>5</sup> is His or Ala;

30 Xaa<sup>11</sup> and Xaa<sup>13</sup> are independently Lys, Arg, or Leu;

Xaa<sup>19</sup> and Xaa<sup>21</sup> are independently Ala or Arg;

Xaa<sup>22-31</sup> is selected from:

35 a)  $\underset{1}{\text{Glu}} \text{ } \underset{5}{\text{Leu}} \text{ } \underset{10}{\text{Leu}} \text{ } \text{Glu} \text{ } \text{Lys} \text{ } \text{Leu} \text{ } \text{Leu} \text{ } \text{Xaa} \text{ } \text{Lys} \text{ } \text{Leu}$  wherein

Xaa is Glu or Arg (SEQ ID NO:26);

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b) Glu Leu Leu Glu Arg Leu Leu Xaa Arg Leu wherein  
 1 5 10

Xaa is Glu, Lys, or Lys(COCH<sub>2</sub>PEGX) and PEGX is a  
 poly(ethylene glycol methyl ether) radical of molecular  
 weight 100 to 10,000 (SEQ ID NO:27);

c) Ala Leu Ala Glu Ala Leu Ala Glu Ala Leu (SEQ ID NO:28);  
 1 5 10

d) Ser Leu Leu Ser Ser Leu Leu Ser Ser Leu (SEQ ID NO:29);  
 1 5 10

e) Ala Phe Tyr Asp Lys Val Ala Glu Lys Leu (SEQ ID NO:30);  
 1 5 10

Xaa<sup>32</sup> is His or Lys;

Xaa<sup>33</sup> is Thr, Glu, or Ala;

Xaa<sup>34</sup> is Ala, hSer, Tyr, or Leu;

and Term is Gly Arg Arg, lactone, OH or NR<sub>2</sub>, where each R is H  
 or (C<sub>1</sub>-C<sub>4</sub>) alkyl; and their pharmaceutically acceptable salts.  
 (Formula I)

A more specific aspect of the invention includes  
 those polypeptides of Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID  
 NO:26), for which <μ<sub>H</sub>> at 100° exceeds 0.45. A still more  
 specific aspect of the invention includes those Formula (I)  
 polypeptides wherein Xaa<sup>22-31</sup> is (SEQ ID NO:26); Xaa<sup>11</sup> and Xaa<sup>13</sup>  
 are both Lys; and Xaa<sup>19</sup> and Xaa<sup>21</sup> are both Arg.

Representative polypeptides include, but are not limited to:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Arg Lys  
 20 25 30



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Leu Lys Glu Leu NH<sub>2</sub> (SEQ ID NO:11).

Another aspect of this invention includes those polypeptides of Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:26); Xaa<sup>11</sup> and Xaa<sup>13</sup> are both Lys; and one of Xaa<sup>19</sup> and Xaa<sup>21</sup> is Arg and the other is Ala. Representative poly-peptides of this subgenus include, but are not limited to:

10 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Ala Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

15 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:12) and

20 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Ala Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

25 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:13).

In another aspect this invention includes those polypeptides of Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:26); one of Xaa<sup>11</sup> and Xaa<sup>13</sup> is Leu and the other is Lys; and Xaa<sup>19</sup> and Xaa<sup>21</sup> are both Arg. Representative poly-peptides of this subgenus include, but are not limited to:

35 Ala Val Ser Glu Ala Gln Leu Leu His Asp Leu Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

40 Leu His Ala Leu OH (SEQ ID NO:14).

In another aspect this invention includes those polypeptides of Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:27), for which  $\langle \mu_H \rangle$  at 100° exceeds 0.50. A further aspect of this invention includes those Formula (I) polypeptides wherein Xaa<sup>22-31</sup> is (SEQ ID NO:27); Xaa<sup>11</sup> and Xaa<sup>13</sup> are both Lys or both Arg; and Xaa<sup>19</sup> and Xaa<sup>21</sup> are both Arg. Representative polypeptides

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of this subgenus include, but are not limited to:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

5 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

Leu His Thr Ala OH (SEQ ID NO:15);

10 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

15 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

Leu His Thr Ala OH (SEQ ID NO:16);

20 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Lys Arg  
20 25 30

25 Leu His Thr Ala OH (SEQ ID NO:17);

30 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu  
20 25

35 Lys(COCH<sub>2</sub>PEG2) Arg Leu His Thr Ala OH (SEQ ID NO:18); and  
30

40 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu  
20 25

45 Lys(COCH<sub>2</sub>PEG5000) Arg Leu His Thr Ala OH (SEQ ID NO:19).  
30

50 In another aspect this invention includes polypeptides of  
Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:28), for which <μ<sub>H</sub>> at  
100° is about 0.25. Representative polypeptides of this  
subgenus include, but are not limited to:

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Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Ala Leu Ala Glu Ala Leu Ala Glu Ala  
 20 25 30  
 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:20).

In another aspect this invention includes polypeptides of  
 Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:29), for which  $\langle\mu_H\rangle$  at  
 100° is about 0.28. Representative polypeptides of this  
 subgenus include, but are not limited to:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Ser Leu Leu Ser Ser Leu Leu Ser Ser  
 20 25 30  
 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:21).

In another aspect this invention includes polypeptides of  
 Formula (I) wherein Xaa<sup>22-31</sup> is (SEQ ID NO:30), for which  $\langle\mu_H\rangle$  at  
 100° is about 0.29. Representative polypeptides of this  
 subgenus include, but are not limited to:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Ala Phe Tyr Asp Lys Val Ala Glu Lys  
 20 25 30  
 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:22).

Still another aspect of this invention includes  
 polypeptide analogs of the physiologically active truncated  
 homolog bPTH(1-34), as shown in Formula (II):

Xaa<sup>1</sup> Val Ser Glu Ile Gln Xaa<sup>7</sup> Xaa<sup>8</sup> His Asn Leu Gly Lys His Leu  
 Xaa<sup>16</sup> Ser Xaa<sup>18</sup> Xaa<sup>19</sup> Arg Xaa<sup>21</sup> Xaa<sup>22-31</sup> His Asn Xaa<sup>34</sup> Term, wherein:

Xaa<sup>1</sup> is Ser or Ala;

Xaa<sup>7</sup> is Leu or Phe;

Xaa<sup>8</sup> is Met or Nle;

Xaa<sup>16</sup> is Asn or Ser;

Xaa<sup>18</sup> is Leu, Met, or Nle;

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Xaa<sup>19</sup> is Glu or Arg;

Xaa<sup>21</sup> is Val or Arg;

Xaa<sup>22-31</sup> is selected from (SEQ ID NO:26, 27, 28, 29, and 30);

Xaa<sup>34</sup> is Phe or Tyr;

5 Term is OH or NR<sub>2</sub>, where each R is H or (C<sub>1</sub>-C<sub>4</sub>)alkyl; and the pharmaceutically acceptable salts thereof. (Formula II)  
Representative polypeptides include, but are not limited to:

10 Ala Val Ser Glu Ile Gln Phe Nle His Asn Leu Gly Lys His Leu  
1 5 10 15

Ser Ser Nle Glu Arg Val Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

15 Leu His Asn Tyr NH<sub>2</sub> (SEQ ID NO:23) and

20 Ala Val Ser Glu Ile Gln Phe Nle His Asn Leu Gly Lys His Leu  
1 5 10 15

Ser Ser Nle Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

25 Leu His Asn Tyr NH<sub>2</sub> (SEQ ID NO:24).

In still another aspect of this invention, it has surprisingly been found that homologs and analogs of PTH and PTHrP having less than 34 amino acids are also potent bone remodeling agents. These compounds are of general formula:

30 Ala Val Ser Glu Xaa<sup>5</sup> Gln Leu Leu His Asp Xaa<sup>11</sup> Gly Xaa<sup>13</sup> Ser Ile  
Gln Asp Leu Xaa<sup>19</sup> Arg Xaa<sup>21</sup> Xaa<sup>22-31</sup> Xaa<sup>32</sup> Xaa<sup>33</sup> Xaa<sup>34</sup> Term,

Representative polypeptides include, but are not limited to:

35 Compound 41: AVSEHQLLHD KGKSIQDLRR RELLEKLLEK LHP-NH<sub>2</sub> (SEQ ID NO:55)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

40 Leu His Pro NH<sub>2</sub> (SEQ ID NO:55).

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Physical Datam.p. 142.8-166.1°C  $[\alpha]_D^{25}$  -53.80 (c 0.38, H<sub>2</sub>O)FAB(C<sub>173</sub>H<sub>295</sub>N<sub>55</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 3929

AAA: Asx	2.0 (2)	Glx	5.7 (6)	Ser	1.8 (2)
His	3.0 (3)	Gly	1.1 (1)	Ala	0.9 (1)
Arg	2.8 (3)	Val	1.2 (1)	Ile	0.9 (1)
Leu	7.4 (8)	Lys	4.4 (4)	Pro	0.9 (1)

Compound 42: AVSEHQLLHD KGKSIQDLRR RELLEKLLEK LP-NH<sub>2</sub> (SEQ ID NO:56)

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1				5					10					15
Gln	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys
				20					25					30

Leu Pro NH<sub>2</sub> (SEQ ID NO:56).

Physical Datam.p. 161.0-177.0°C  $[\alpha]_D^{25}$  -61.97 (c 0.19, H<sub>2</sub>O)FAB(C<sub>167</sub>H<sub>288</sub>N<sub>52</sub>O<sub>48</sub>): [M+H]<sup>+</sup> 3792.0

AAA: Asx	2.2 (2)	Glx	5.9 (6)	Ser	1.9 (2)
His	2.1 (2)	Gly	1.1 (1)	Ala	1.0 (1)
Arg	3.0 (3)	Val	1.1 (1)	Ile	1.0 (1)
Leu	7.9 (8)	Lys	4.3 (4)	Pro	0.9 (1)

The skilled artisan will appreciate that numerous permutations of the polypeptide analogs may be synthesized which will possess the desirable attributes of those described herein provided that an amino acid sequence having a mean hydrophobic moment per residue at 100° ± 20° greater than about 0.20 is inserted at positions (22-31).

Classical Synthesis of the Polypeptides

The polypeptides of the instant invention may be synthesized by methods such as those set forth in J.M. Stewart and J.D. Young, *Solid Phase Peptide Synthesis*, 2nd ed., Pierce Chemical Co., Rockford, Illinois (1984) and J. Meienhofer, *Hormonal Proteins and Peptides*, Vol. 2, Academic

Press, New York, (1973) for solid phase synthesis and E. Schroder and K. Lubke, *The Peptides*, Vol. 1, Academic Press, New York, (1965) for solution synthesis.

In general, these methods involve the sequential addition of protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid and any reactive side chain group are protected. This protected amino acid is then either attached to an inert solid support, or utilized in solution, and the next amino acid in the sequence, also suitably protected, is added under conditions amenable to formation of the amide linkage. After all the desired amino acids have been linked in the proper sequence, protecting groups and any solid support are removed to afford the crude polypeptide. The polypeptide is desalted and purified, preferably chromatographically, to yield the final product.

A preferred method of preparing the analogs of the physiologically active truncated polypeptides, having fewer than about forty amino acids, involves solid phase peptide synthesis. In this method the  $\alpha$ -amino ( $N^\alpha$ ) functions and any reactive side chains are protected by acid- or base-sensitive groups. The protecting group should be stable to the conditions of peptide linkage formation, while being readily removable without affecting the extant polypeptide chain. Suitable  $\alpha$ -amino protecting groups include, but are not limited to *t*-butoxycarbonyl (Boc), benzyloxycarbonyl (Cbz), *o*-chlorobenzyloxycarbonyl, biphenylisopropylloxycarbonyl, *t*-amyloxycarbonyl (Amoc), isobornylloxycarbonyl,  $\alpha,\alpha$ -dimethyl-3,5-dimethoxybenzyloxy-carbonyl, *o*-nitrophenylsulfenyl, 2-cyano-*t*-butoxycarbonyl, 9-fluorenylmethoxycarbonyl (Fmoc) and the like, preferably *t*-butoxycarbonyl (Boc). Suitable side chain protecting groups include, but are not limited to: acetyl, benzyl (Bzl), benzyloxymethyl (Bom), *o*-bromobenzyloxycarbonyl, *t*-butyl, *t*-butyldimethylsilyl, 2-chlorobenzyl (Cl-z), 2,6-dichlorobenzyl, cyclohexyl, cyclopentyl, isopropyl, pivalyl, tetrahydropyran-2-yl, tosyl (Tos), trimethylsilyl,

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and trityl.

In solid phase synthesis, the C-terminal amino acid is first attached to a suitable resin support. Suitable resin supports are those materials which are inert to the reagents and reaction conditions of the stepwise condensation and deprotection reactions, as well as being insoluble in the media used. Examples of commercially available resins include styrene/divinylbenzene resins modified with a reactive group, e.g., chloromethylated co-poly-(styrene-divinylbenzene), hydroxymethylated co-poly-(styrene-divinylbenzene), and the like. Benzylated, hydroxy-methylated phenylacetamidomethyl (PAM) resin is preferred. When the C-terminus of the compound is an amide, a preferred resin is p-methylbenzhydrylamino-co-poly(styrene-divinyl-benzene) resin.

Attachment to the PAM resin may be accomplished by reaction of the N<sup>α</sup>-protected amino acid, preferably the Boc-amino acid, as its ammonium, cesium, triethylammonium, 1,5-diazabicyclo-[5.4.0]undec-5-ene, tetramethylammonium, or similar salt in ethanol, acetonitrile, N,N-dimethylformamide (DMF), and the like, preferably the cesium salt in DMF, with the resin at an elevated temperature, for example between about 40° and 60°C, preferably about 50°C, for from about 12 to 72 hours, preferably about 48 hours.

The N<sup>α</sup>-Boc-amino acid may be attached to the benzhydrylamine resin by means of, for example, an N,N'-diisopropylcarbodiimide (DIC)/1-hydroxybenzotriazole (HOBt) mediated coupling for from about 2 to about 24 hours, preferably about 2 hours at a temperature of between about 10° and 50°C, preferably 25°C in a solvent such as dichloromethane or dimethylformamide, preferably dichloromethane.

The successive coupling of protected amino acids may be carried out by methods well known in the art, typically in an automated peptide synthesizer. Following neutralization with triethylamine or similar base, each protected amino acid is preferably introduced in approximately 1.5 to 2.5 fold molar excess and the coupling carried out in an inert, nonaqueous, polar solvent such as dichloromethane, DMF, or mixtures



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thereof, preferably in dichloromethane at ambient temperature. Representative coupling agents are

5 N,N'-dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide (DIC) or other carbodiimide, either alone or in the presence of 1-hydroxybenzotriazole (HOBt), O-acyl ureas, benzotriazol-1-yl-oxytris(pyrrolidino)phosphonium  
10 hexafluorophosphate (PyBop), N-hydroxysuccinimide, other N-hydroxyimides, or oximes. Alternatively, protected amino acid active esters (e.g. p-nitrophenyl, pentafluorophenyl and the like) or symmetrical anhydrides may be used.

At the end of the solid phase synthesis the fully  
protected peptide is removed from the resin. When the linkage  
to the resin support is of the benzyl ester type, cleavage may  
be effected by means of aminolysis with an alkylamine or  
15 fluoroalkylamine for peptides with an alkylamide C-terminus, or by aminolysis with, for example, ammonia/methanol or ammonia/ethanol for peptides with an unsubstituted amide C-terminus, at a temperature between about -10° and 50°C, preferably about 25°C, for between about 12 and 24 hours,  
20 preferably about 18 hours. Peptides with a hydroxy C-terminus may be cleaved by HF or other strongly acidic deprotection regimen or by saponification. Alternatively, the peptide may be removed from the resin by transesterification, e.g., with methanol, followed by aminolysis or saponification. The  
25 protected peptide may be purified by silica gel chromatography.

The side chain protecting groups may be removed from the peptide by treating the aminolysis product with, for example, anhydrous liquid hydrogen fluoride in the presence of anisole  
30 or other carbonium ion scavenger, treatment with hydrogen fluoride/pyridine complex, treatment with tris(trifluoroacetyl)boron and trifluoroacetic acid, by reduction with hydrogen and palladium on carbon or polyvinylpyrrolidone, or by reduction with sodium in liquid  
35 ammonia, preferably with liquid hydrogen fluoride and anisole at a temperature between about -10° and +10°C, preferably at about 0°C, for between about 15 minutes and 2 hours,

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preferably about 1.5 hours. For peptides on the benzhydrylamine resin, the resin cleavage and deprotection steps may be combined in a single step utilizing liquid hydrogen fluoride and anisole as described above.

5 The solution may be desalted (e.g. with BioRad AG-3<sup>®</sup> anion exchange resin) and the peptide purified by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on underivatized  
10 co-poly(styrene-divinylbenzene), e.g. Amberlite<sup>®</sup> XAD; silica gel adsorption chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, e.g. on Sephadex<sup>®</sup> G-25; countercurrent distribution; or high  
15 performance liquid chromatography (HPLC), especially reversed-phase HPLC on octyl- or octadecylsilylsilica (ODS) bonded phase column packing.

Thus, another aspect of the present invention relates to processes for preparing polypeptides and pharmaceutically acceptable salts thereof which processes comprise sequentially  
20 condensing protected amino acids on a suitable resin support, removing the protecting groups and resin support, and purifying the product, to afford analogs of the physiologically active truncated homologs and analogs of PTH and PTHrp, preferably of PTH(1-34) and PTHrp(1-34), in which  
25 the amino acids at positions (22-31) form an amphipathic  $\alpha$ -helical peptide sequence, as defined above.

#### Recombinant Synthesis of the Polypeptides

Alternatively, the polypeptides of this invention may be  
30 prepared by cloning and expression of a gene encoding for the desired polypeptide. In this process, a plasmid containing the desired DNA sequence is prepared and inserted into an appropriate host microorganism, typically a bacteria, such as *E. coli*, or a yeast, such as *Saccharomyces cerevisiae*,  
35 inducing the host microorganism to produce multiple copies of the plasmid, and so of the cDNA encoding for the polypeptide analogs of this invention.

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First, a synthetic gene coding for the selected PTH or PTHrp analog is designed with convenient restriction enzyme cleavage sites to facilitate subsequent alterations. Polymerase chain reaction (PCR), as taught by Mullis in U. S. Patent Nos. 4,683,195 and 4,683,202, may be used to amplify the sequence.

The amplified synthetic gene may be isolated and ligated to a suitable plasmid, such as a Trp LE plasmid, into which four copies of the gene may be inserted in tandem. Preparation of Trp LE plasmids is described in U.S. Patent No. 4,738,921 and European Patent Publication No. 0212532. Trp LE plasmids generally produce 8-10 times more protein than Trp E plasmids. The multi-copy gene may then be expressed in an appropriate host, such as *E. coli* or *S. cerevisiae*.

The specific expression vector used herein was Trp LE 18 Prot (Ile<sup>3</sup>, Pro<sup>5</sup>) containing the following elements: a pBR322 fragment (EcoRI-BamHI) containing the ampicillin resistant gene and the plasmid origin of replication; an EcoRI-SacII fragment containing the trp promoter and the trpE gene; an HIV protease (Ile<sup>3</sup>,Pro<sup>5</sup>) gene fragment (SacII-HindIII); a bGRF gene fragment (HindIII-BamHI); and a transcription terminator from *E. coli* rpoC gene. The HIV protease and bGRF gene fragments are not critical and may be replaced with other coding sequences, if desired.

The expressed multimeric fusion proteins accumulate intracellularly into stable inclusion bodies and may be separated by centrifugation from the rest of the cellular protein. The isolated fusion protein is converted to the monomeric PTH or PTHrp analog and may be purified by cation exchange and/or reverse phase HPLC.

Alternative methods of cloning, amplification, expression, and purification will be apparent to the skilled artisan. Representative methods are disclosed in Maniatis, et al., *Molecular Cloning, a Laboratory Manual*, 2nd Ed., Cold Spring Harbor Laboratory (1989).

Utility and Administration

The polypeptides of this invention are useful for the prevention and treatment of a variety of mammalian conditions manifested by loss of bone mass. In particular, the compounds of this invention are indicated for the prophylaxis and therapeutic treatment of osteoporosis and osteopenia in humans.

In general, the polypeptides of this invention, or salts thereof, are administered in amounts between about 0.002 and 10  $\mu\text{g}/\text{kg}$  body weight per day, preferably from about 0.04 to about 0.2  $\mu\text{g}/\text{kg}$  body weight per day. For a 50 kg human female subject, the daily dose of active ingredient is from about 0.1 to about 500  $\mu\text{g}$ s, preferably from about 2.0 to about 100  $\mu\text{g}$ s. In other mammals, such as horses, dogs, and cattle, higher doses may be required. This dosage may be delivered in a conventional pharmaceutical composition by a single administration, by multiple applications, or via controlled release, as needed to achieve the most effective results, preferably one or more times daily by injection.

The selection of the exact dose and composition and the most appropriate delivery regimen will be influenced by, *inter alia*, the pharmacological properties of the selected polypeptide, the nature and severity of the condition being treated, and the physical condition and mental acuity of the recipient.

In the treatment of corticosteroid induced osteopenia, it is expected that the requisite dose of polypeptide will be greater for higher doses of corticosteroids.

Representative delivery regimens include oral, parenteral (including subcutaneous, intramuscular and intravenous), rectal, buccal (including sublingual), pulmonary, transdermal, and intranasal.

Pharmaceutically acceptable salts retain the desired biological activity of the parent polypeptide without toxic side effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric

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acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pamoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalene disulfonic acids, polygalacturonic acid and the like; (b) base addition salts formed with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, and the like; or with an organic cation formed from N,N'-dibenzylethylenediamine or ethylenediamine; or (c) combinations of (a) and (b), e.g., a zinc tannate salt and the like.

A further aspect of the present invention relates to pharmaceutical compositions comprising as an active ingredient a polypeptide of the present invention, or pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable, non-toxic carrier. As mentioned above, such compositions may be prepared for parenteral (subcutaneous, intramuscular or intravenous) administration, particularly in the form of liquid solutions or suspensions; for oral or buccal administration, particularly in the form of tablets or capsules; for pulmonary or intranasal administration, particularly in the form of powders, nasal drops or aerosols; and for rectal or transdermal administration.

The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, PA., (1985). Formulations for parenteral administration may contain as excipients sterile water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. For oral administration, the formulation can be enhanced by the addition of bile salts or acylcarnitines. Formulations for nasal administration may be solid and may contain excipients,

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for example, lactose or dextran, or may be aqueous or oily solutions for use in the form of nasal drops or metered spray. For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

When formulated for nasal administration, the absorption across the nasal mucous membrane may be enhanced by surfactant acids, such as for example, glycocholic acid, cholic acid, taurocholic acid, ethocholic acid, deoxycholic acid, chenodeoxycholic acid, dehydrocholic acid, glycodeoxycholic acid, cyclodextrins and the like in an amount in the range between about 0.2 and 15 weight percent, preferably between about 0.5 and 4 weight percent, most preferably about 2 weight percent.

Delivery of the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year, may be accomplished by a single administration of a controlled release system containing sufficient active ingredient for the desired release period. Various controlled release systems, such as monolithic or reservoir-type microcapsules, depot implants, osmotic pumps, vesicles, micelles, liposomes, transdermal patches, iontophoretic devices and alternative injectable dosage forms may be utilized for this purpose. Localization at the site to which delivery of the active ingredient is desired is an additional feature of some controlled release devices, which may prove beneficial in the treatment of certain disorders.

One form of controlled release formulation contains the polypeptide or its salt dispersed or encapsulated in a slowly degrading, non-toxic, non-antigenic polymer such as copoly(lactic/glycolic) acid, as described in the pioneering work of Kent, Lewis, Sanders, and Tice, U.S. 4,675,189. The compounds or, preferably, their relatively insoluble salts, may also be formulated in cholesterol or other lipid matrix pellets, or silastomer matrix implants. Additional slow release, depot implant or injectable formulations will be

apparent to the skilled artisan. See, for example, *Sustained and Controlled Release Drug Delivery Systems*, J. R. Robinson ed., Marcel Dekker, Inc., New York, 1978, and R. W. Baker, *Controlled Release of Biologically Active Agents*, John Wiley & Sons, New York, 1987.

#### Nasal Delivery Systems

Commercially available spray pumps and bottles were used to deliver the nasal solutions for evaluation in male cynomolgous monkeys. Spray pumps with delivery volumes of 50 and 100  $\mu$ l were obtained from Valois. For the initial studies, adult size spray tips were used. For later studies, pediatric size spray tips were used. Size of the tips did not affect the spray pump's performance. Samples from each lot of spray pumps were tested to determine the number of actuations required to prime the pumps, the accuracy of their delivery volumes, and the consistency of their spray patterns.

Monkeys were anesthetized prior to dosing and reclined on their backs when dosed. To deliver 4 mg doses of peptide, one 100  $\mu$ l spray of 100 mg/ml solution was dosed in each nostril. To deliver 1 mg doses of peptide, one 50  $\mu$ l spray of 20 mg/ml solution was dosed in one nostril. Each monkey was dosed subcutaneously with 50  $\mu$ g of peptide in a subsequent study to serve as its own control. Nasal bioavailability was calculated relative to the subcutaneous injection. Blood samples were drawn 0, 0.25, 0.5, 1, 2, 4, 6, and 8 hours post dosing and assayed for peptide by RIA. Pharmacokinetic parameters were calculated from the plasma profiles. Area under the curve (AUC) of the plasma concentration profile was calculated by the trapezoid rule, and bioavailability determined relative to the average AUC from s.c. dosing for all animals.

Average bioavailabilities approaching 50% were obtained for the compositions of this invention. The T<sub>max</sub> for the nasal formulations was between 0.3 and 0.7 hours, similar to the T<sub>max</sub> measured for s.c. administration (0.6 hrs.).

Among the bile acid surfactants useful in this invention

are glycocholic acid, cholic acid, taurocholic acid, cholanic acid, ethocholic acid, desoxycholic acid, chenodesoxycholic acid, and their pharmaceutically acceptable salts, including the sodium, potassium, ammonium, calcium, and magnesium salts as well as other non-toxic organic and inorganic cations. Preferably, the bile acid surfactant is an alkali metal salt of glycocholic or taurocholic acid, most preferably the sodium salt.

Other materials, such as preservatives, salts to achieve isotonicity, buffers, and the like, may be added to the nasal formulations. Typically, the peptide is present in an amount from about 1 mg/ml to about 100 mg/ml and the absorption enhancer concentration is from about 0.5 mg/ml to about 50 mg/ml.

The following specific Examples are intended to illustrate the synthesis and testing of representative compounds of the invention and should not be construed as limiting the scope of the claims. In the Examples, "m. p." is melting point, " $[\alpha]_D^{25}$ " is the optical activity at 25°C at the given concentration in the indicated solvent, "FAB" is fast atom bombardment mass spectrometry, and "AAA" is amino acid analysis, with expected values in parentheses following the observed values. The amino acid analyses were conducted on a Hewlett-Packard AminoQuant Analyzer following the manufacturer's recommended protocols. Primary amino acids were derivatized with o-phthalaldehyde; secondary amino acids with Fmoc. Fluorescent detection of the derivatized amino acids was used for quantification. The protected amino acids were obtained from Applied Biosystems Inc. (Foster City, CA).

#### EXAMPLE I

Compound 1 (SEQ ID NO:7) was prepared on 0.5 mmol scale by the solid phase method on 4-methylbenzhydrylamine resin, using an automated Applied Biosystems Model 430A peptide synthesizer. The  $\alpha$ -amino groups were protected with t-butoxycarbonyl (Boc). The side chain protecting groups were: benzyl (Bzl) for Asp, Glu, and Ser; tosyl (Tos) for Arg; benzyloxymethyl (Bom) for His; and 2-chlorobenzyl (Cl-z) for



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Lys. The amino acids were coupled sequentially using N,N-dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt) following Stewart and Young (*supra*). After each amino acid coupling, the peptide was acetylated using a mixture of acetic anhydride and diisopropylethylamine in N-methylpyrrolidone. The completed peptide was cleaved from the resin with simultaneous deprotection of the side chain protecting groups using anhydrous HF (25 mL) in the presence of anisole (2.5 mL) at -10°C for 30 minutes and at 0°C for 60 minutes. After evaporation of the HF *in vacuo*, the residue was washed with anhydrous ether, and the crude peptide extracted with 10% acetic acid. Lyophilization of the 10% acetic acid extract gave 900 mgs of crude product. The peptide was purified by medium pressure ODS reversed phase column chromatography using a gradient of 22-45% CH<sub>3</sub>CN in 0.1% TFA. The product eluted in three fractions, which were concentrated and lyophilized to give 130 mgs of white solid of >98% purity.

Compound 1:AVSEHOLLHDKGKSIODLRRRELLEKLLEKLHTA-NH<sub>2</sub> (SEQ ID NO:7)

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1				5					10					15
Gln	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys
			20						25					30
Leu His Thr Ala NH <sub>2</sub> (SEQ ID NO:7)														

Physical Data: m.p. 150-159°C      [α]<sub>D</sub><sup>25</sup> -34.88° (c 0.16, H<sub>2</sub>O)  
 FAB (C<sub>175</sub>H<sub>300</sub>N<sub>56</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4005.5  
 AAA: Asp, 1.9(2); Glu, 5.6(6); Ser, 1.6(2); His, 2.7(3); Gly, 1.0(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 2.8(3); Val, 1.0(1); Ile, 0.9(1); Leu, 7.3(8); Lys, 4.0(4).

Similarly, Compounds 2, 5-18, 21-27, 29-36, 38-48, 50-54, 58-64 and 66-70 were prepared and characterized, substituting PAM resin as needed for the synthesis of hydroxy-terminal polypeptides.

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Compound 2:

AVSEHQLLHDKGKSIQDLRRRELLEKLLEKLHTA-OH (SEQ ID NO:6)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

10 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Ala OH (SEQ ID NO:6)

15 Physical Data: m.p. 154-170°C  $[\alpha]_D^{25}$  -49.35° (c 0.46, H<sub>2</sub>O)  
FAB (C<sub>175</sub>H<sub>301</sub>N<sub>57</sub>O<sub>50</sub>): [M+H]<sup>+</sup> 4005.0  
AAA: Asp, 2.1(2); Glu, 5.9(6); Ser, 1.7(2); His, 2.9(3); Gly  
1.1(1); Thr, 1.0(1); Ala, 1.9(2); Arg, 3.0(3); Val,  
1.2(1); Ile, 1.0(1); Leu, 7.8(8); Lys, 4.2(4).

Compound 5:

AVSEHOLLHDKGKSIQDLRRRELLERLLERLHTA-OH (SEQ ID NO:15)

25 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

30 Leu His Thr Ala OH (SEQ ID NO:15)

35 Physical Data: m.p. 147-165°C  $[\alpha]_D^{25}$  -49.17° (c 0.66, H<sub>2</sub>O)  
FAB (C<sub>175</sub>H<sub>299</sub>N<sub>59</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4061  
AAA: Asp, 2.1(2); Gly, 6.1(6); Ser, 1.8(2); His, 3.1(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 5.0(5); Val,  
1.0(1); Ile, 0.9(1); Leu, 7.7(8); Lys, 1.9(2).

Compound 6:

AVSEHOLLHDRGRSIODLRRRELLERLLERLHTA-OH (SEQ ID NO:16)

45 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

50 Leu His Thr Ala OH (SEQ ID NO:16)

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Physical Data: m.p. 150-170°C  $[\alpha]_D^{25}$  -48.65° (c 0.54, H<sub>2</sub>O)  
 FAB (C<sub>175</sub>H<sub>299</sub>N<sub>63</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4118.0  
 AAA: Asp, 2.1(2); Glu, 6.1(6); Ser, 1.8(2); His, 3.2(3); Gly,  
 1.2(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 6.9(7); Val,  
 1.0(1); Ile, 1.0(1); Leu, 7.8(8).

Compound 7:

AVSEHOLLHDRGRSIODLRRRELLERLLKRLHTA-OH (SEQ ID NO:17)

Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Lys Arg  
 20 25 30

Leu His Thr Ala OH (SEQ ID NO:17)

Physical Data: m.p. 177-182°C  $[\alpha]_D^{25}$  -46.17° (c 0.14, H<sub>2</sub>O)  
 FAB (C<sub>176</sub>H<sub>304</sub>N<sub>64</sub>O<sub>50</sub>): [M+H]<sup>+</sup> 4117  
 AAA: Asp, 2.0(2); Glu, 4.8(5); Ser, 1.8(2); His, 3.2(3); Gly,  
 1.1(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 6.7(7); Val,  
 1.0(1); Ile, 1.0(1); Lys, 7.7(8); Lys, 0.9(1).

Compound 8:

AVSEHOLLHDKGKSIODLRRRELLEKLLRKLHTA-OH (SEQ ID NO:5)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Arg Lys  
 20 25 30

Leu His Thr Ala OH (SEQ ID NO:5)

Physical Data: m.p. 147-165°C  $[\alpha]_D^{25}$  -49.17° (c 0.66, H<sub>2</sub>O)  
 FAB (C<sub>176</sub>H<sub>305</sub>N<sub>59</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4033.0  
 AAA: Asp, 2.0(2); Glu, 4.8(5); Ser, 1.8(2); His, 2.7(3); Gly,  
 1.1(1); Thr, 0.9(1); Ala, 2.0(2); Arg, 3.9(4); Val,  
 1.0(1); Ile, 1.0(1); Leu, 7.9(8); Lys, 4.0(4).

Compound 9:

AVSEHOLLHDKGKSIODLRRRELLEKLLKLTAGRR-OH (SEQ ID NO:10)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

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Leu His Thr Ala Gly Arg Arg OH (SEQ ID NO:10)

Physical Data: m.p. 158-160°C  $[\alpha]_D^{25}$  -44.76° (c 0.1, H<sub>2</sub>O)  
 FAB (C<sub>189</sub>H<sub>326</sub>N<sub>64</sub>O<sub>55</sub>): [M]<sup>+</sup> 4375.0  
 AAA: Asp, 2.0(2); Glu, 5.9(6); Ser, 1.7(2); His, 2.9(3); Gly,  
 2.3(2); Thr, 1.0(1); Ala, 1.9(2); Arg, 5.0(5); Val,  
 1.2(1); Ile, 1.0(1); Leu, 7.8(8); Lys, 4.3(4).

Compound 10:AVSEAQLLHDLGKSIODLRRELLEKLLEKLHAL-OH (SEQ ID NO:14)

Ala Val Ser Glu Ala Gln Leu Leu His Asp Leu Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Ala Leu OH (SEQ ID NO:14)

Physical Data: m.p. 170-175°C  $[\alpha]_D^{25}$  -31.59° (c 0.54, H<sub>2</sub>O)  
 FAB (C<sub>174</sub>H<sub>300</sub>N<sub>52</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 3936.0  
 AAA: Asp, 2.0(2); Glu, 6.0(6); Ser, 1.8(2); His, 2.0(2); Gly,  
 1.2(1); Ala, 3.0(3); Arg, 2.8(3); Val, 1.1(1); Ile,  
 1.0(1); Leu, 9.9(10); Lys, 3.0(3).

Compound 11:AVSEHOLLHDKGKSIODLRRELLEKLLELLKEL-NH<sub>2</sub> (SEQ ID NO:11)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu Lys Glu Leu NH<sub>2</sub> (SEQ ID NO:11)

Physical Data: m.p. 172-174°C  $[\alpha]_D^{25}$  -43.29° (c 0.2, H<sub>2</sub>O)  
 FAB (C<sub>179</sub>H<sub>311</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4065.8  
 AAA: Asp, 2.2(2); Glu, 7.7(7); Ser, 1.7(2); His, 2.0(2); Gly,  
 1.0(1); Ala, 1.0(1); Arg, 3.0(3); Val, 1.1(1); Ile,  
 1.0(1); Leu, 9.3(9); Lys, 5.1(5).

Compound 12:AVSEIOFXHNLGKHLSSXERVELLEKLLEKLHNY-NH<sub>2</sub> (X=Nle, SEQ ID NO:23)

Ala Val Ser Glu Ile Gln Phe Nle His Asn Leu Gly Lys His Leu  
 1 5 10 15

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Ser Ser Nle Glu Arg Val Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Asn Tyr NH<sub>2</sub> (SEQ ID NO:23)

5 Physical Data: m.p. 178°C [α]<sub>D</sub><sup>25</sup> -36.88° (c 0.4, H<sub>2</sub>O)  
FAB (C<sub>182</sub>H<sub>295</sub>N<sub>50</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4001.6  
10 AAA: Asp, 2.1(2); Glu, 6.5(6); Ser, 2.7(3); His, 3.1(3); Gly,  
1.1(1); Ala, 1.0(1); Arg, 1.0(1); Tyr, 0.8(1); Val,  
2.0(2); Phe, 1.0(1); Ile, 0.9(1); Leu+Nle, 8.5(7+2); Lys,  
3.1(3).

Compound 13:

15 AVSEIOFXHNLGKHLSSXRRRELLEKLLEKLHNY-NH<sub>2</sub> (X=Nle, SEQ ID NO:24)

Ala Val Ser Glu Ile Gln Phe Nle His Asn Leu Gly Lys His Leu  
1 5 10 15

20 Ser Ser Nle Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Asn Tyr NH<sub>2</sub> (SEQ ID NO:24)

25 Physical Data: m.p. 260°C [α]<sub>D</sub><sup>25</sup> -37.02° (c 0.2, H<sub>2</sub>O)  
FAB (C<sub>184</sub>H<sub>304</sub>N<sub>56</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4084  
30 AAA: Asp, 2.1(2); Glu, 5.5(5); Ser, 2.6(3); His, 3.1(3); Ala,  
1.0(1); Gly, 1.1(1); Arg, 3.2(3); Tyr, 1.0(1); Val,  
1.0(1); Phe, 1.0(1); Ile, 1.0(1); Leu, 9.0(9); Lys,  
3.0(3).

Compound 14:

35 AVSEHOLLHDKGKSIODLRRRALAEALAEALHTA-NH<sub>2</sub> (SEQ ID NO:20)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

40 Gln Asp Leu Arg Arg Arg Ala Leu Ala Glu Ala Leu Ala Glu Ala  
20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:20)

45 Physical Data: m.p. 190-225°C [α]<sub>D</sub><sup>25</sup> -56.58° (c 0.36, H<sub>2</sub>O)  
FAB (C<sub>161</sub>H<sub>272</sub>N<sub>54</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 3747.0  
50 AAA: Asp, 2.1(2); Glu, 4.9(5); Ser, 1.7(2); His, 2.6(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 7.6(7); Arg, 2.8(3); Val,  
1.2(1); Ile, 1.0(1); Leu, 6.6(6); Lys, 1.9(2).

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Compound 15:AVSEHOLLHDKGKSIODLARRELLEKLLEKLHTA-NH<sub>2</sub> (SEQ ID NO:12)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Ala Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

10 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:12)

Physical Data: m.p. 170-180°C  $[\alpha]_D^{25}$  -48.19° (c 0.2, H<sub>2</sub>O)

FAB (C<sub>172</sub>H<sub>293</sub>N<sub>53</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 3919.0

15 AAA: Asp, 2.1(2); Glu, 6.1(6); Ser, 1.7(2); His, 3.1(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 3.0(3); Arg, 2.1(2); Val,  
1.1(1); Ile, 1.0(1); Leu, 8.0(8); Lys, 4.4(4).

20 Compound 16:

AVSEHOLLHDKGKSIODLRRRAELLEKLLEKLHTA-NH<sub>2</sub> (SEQ ID NO:13)

25 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Ala Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

30 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:13)

Physical Data: m.p. 190-195°C  $[\alpha]_D^{25}$  -50.50° (c 0.4, H<sub>2</sub>O)

FAB (C<sub>172</sub>H<sub>293</sub>N<sub>53</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 3919.0

35 AAA: Asp, 2.1(2); Glu, 6.0(6); Ser, 1.8(2); His, 3.1(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 3.0(3); Arg, 2.1(2); Val,  
1.1(1); Ile, 1.0(1); Leu, 7.5(8); Lys, 4.2(4).

Compound 17:

40 AVSEHOLLHDKGKSIODLRRRSLLSSLSSLHTA-NH<sub>2</sub> (SEQ ID NO:21)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

45 Gln Asp Leu Arg Arg Arg Ser Leu Leu Ser Ser Leu Leu Ser Ser  
20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:21)

Physical Data: m.p. 195-204°C  $[\alpha]_D^{25}$  -67.11° (c 0.3, H<sub>2</sub>O)

50 FAB (C<sub>163</sub>H<sub>280</sub>N<sub>54</sub>O<sub>50</sub>): [M+H]<sup>+</sup> 3796.0

AAA: Asp, 2.1(2); Glu, 2.9(3); Ser, 6.8(7); His, 3.1(3); Gly,  
1.2(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.0(3); Val,  
1.0(1); Ile, 1.0(1); Leu, 8.2(8); Lys, 2.0(2).

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Compound 18:AVSEHOLLHDKGKSIODLRRRAFYDKVAEKLHTA-NH<sub>2</sub> (SEQ ID NO:22)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Ala Phe Tyr Asp Lys Val Ala Glu Lys  
 20 25 30  
 10 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:22)

Physical Data: m.p. 200-207°C  $[\alpha]_D^{25}$  -60.26° (c 0.6, H<sub>2</sub>O)  
 FAB (C<sub>174</sub>H<sub>284</sub>N<sub>56</sub>O<sub>50</sub>): [M+H]<sup>+</sup> 3960.0  
 15 AAA: Asp, 2.9(3); Glu, 3.5(4); Ser, 1.4(2); His, 2.6(3); Gly,  
 0.9(1); Thr, 1.0(1); Ala, 4.0(4); Arg, 3.0(3); Tyr,  
 0.9(1); Val, 1.9(2); Phe, 1.1(1); Ile, 0.9(1); Leu,  
 3.6(4); Lys, 4.1(4).

Compound 21:AVSEIOFLHN LGKHLSSLRR RELLEKLLEK LHNH-NH<sub>2</sub> (SEQ ID NO:35)

25 Ala Val Ser Glu Ile Gln Phe Leu His Asn Leu Gly Lys His Leu  
 1 5 10 15  
 Ser Ser Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 30 Leu His Asn Tyr NH<sub>2</sub> (SEQ ID NO:35)

Physical Data: m.p. 148-155°C  $[\alpha]_D^{25}$  -45.97 (c 0.26, H<sub>2</sub>O)  
 FAB (C<sub>184</sub>H<sub>304</sub>N<sub>56</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4084  
 35 AAA: Asx, 2.1(2); Glx, 5.0(5); Ser, 2.7(3); His, 3.0(3); Gly,  
 1.0(1); Ala, 0.9(1); Arg, 3.1(3); Tyr, 0.9(1); Val,  
 1.0(1); Phe, 0.9(1); Ile, 0.9(1); Leu 9.3(9); Lys,  
 3.2(3).

Compound 24:AVSEHOLLHD KGKSIODLKL KELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:38)

45 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Lys Leu Lys Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 50 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:38)

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Physical Data: m.p. 175-182°C  $[\alpha]_D^{25}$  -49.99(c 0.47, H<sub>2</sub>O)  
 FAB(C<sub>175</sub>H<sub>299</sub>N<sub>49</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 3906.5  
 AAA: Asx, 2.1(2); Glx, 6.5(6); Ser, 1.8(2); His, 3.1(3); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.1(2); Val, 1.1(1); Ile,  
 1.0(1); Leu, 9.1(9); Lys, 6.5(6).

Compound 25:

AVSEHOLLHD KGKSIODLRR RELLERLLER LHTA-NH<sub>2</sub> (SEQ ID NO:39)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
 20 25 30

Leu His Thr Ala-NH<sub>2</sub> (SEQ ID NO:39)

Physical Data: m.p. 136.5-153.5°C  $[\alpha]_D^{25}$  -32.57(c 0.13, H<sub>2</sub>O)  
 FAB(C<sub>175</sub>H<sub>300</sub>N<sub>60</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4060.8  
 AAA: Asx, 2.2(2); Glx, 6.2(6); Ser, 1.8(2); His, 3.2(3); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.1(2); Arg, 5.2(5); Val,  
 1.1(1); Ile, 1.1(1); Leu, 8.4(8); Lys, 2.2(2).

Compound 26:

AVSEHOLLHD KGKSIODLRR RELLERLLER LHTAP-OH (SEQ ID NO:40)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
 20 25 30

Leu His Thr Ala Pro OH (SEQ ID NO:40)

Physical Data: m.p. 125.8-127.2°C  $[\alpha]_D^{25}$  -54.62(c 0.23, H<sub>2</sub>O)  
 FAB(C<sub>180</sub>H<sub>306</sub>N<sub>60</sub>O<sub>53</sub>): [M+H]<sup>+</sup> 4158.0  
 AAA: Asx, 2.1(2); Glx, 6.2(6); Ser, 1.8(2); His, 2.9(3); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 5.1(5); Val,  
 1.0(1); Ile, 1.0(1); Leu, 8.0(8); Lys, 2.1(2); Pro,  
 1.1(1).

Compound 27:

AVSEHOLLHD KGKSIODLRR RELLERLLER LHTAGRR-OH (SEQ ID NO:41)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15



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Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

Leu His Thr Ala Gly Arg Arg OH (SEQ ID NO:41)  
35

Physical Data: m.p. 106-137.3°C  $[\alpha]_D^{25}$  -39.55 (c 0.67, H<sub>2</sub>O)  
FAB(C<sub>189</sub>H<sub>326</sub>N<sub>68</sub>O<sub>55</sub>): [M+H]<sup>+</sup> 4430.5  
AAA: Asx, 2.1(2); Glx, 5.9(6); Ser, 1.6(2); His, 2.7(3); Gly,  
2.2(2); Thr, 1.0(1); Ala, 1.8(2); Arg, 7.3(7); Val,  
0.8(1); Ile, 1.0(1); Leu, 8.1(8); Lys, 2.1(2).

Compound 29:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTY-NH<sub>2</sub> (SEQ ID NO:43)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Tyr NH<sub>2</sub> (SEQ ID NO:43)

Physical Data: m.p. 160-172°C  $[\alpha]_D^{25}$  -49.85 (c 0.34, H<sub>2</sub>O)  
FAB(C<sub>181</sub>H<sub>304</sub>N<sub>56</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4096.9  
AAA: Asx, 2.0(2); Glx, 5.6(6); Ser, 1.7(2); His, 3.1(3); Gly,  
1.1(1); Thr, 0.9(1); Ala, 0.9(1); Arg, 3.0(3); Tyr,  
0.9(1); Val, 1.0(1); Ile, 1.0(1); Leu, 7.7(8); Lys,  
4.4(4).

Compound 30:

AVSEHOLLHD KGYSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:44)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Tyr Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:44)

Physical Data: m.p. 130-171°C  $[\alpha]_D^{25}$  -40.65 (c 0.34, H<sub>2</sub>O)  
FAB(C<sub>178</sub>H<sub>297</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4039.4  
AAA: Asx, 2.0(2); Glx, 5.5(6); Ser, 1.8(2); His, 3.4(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 2.9(3); Tyr,  
0.8(1); Val, 1.0(1); Ile, 0.9(1); Leu, 7.9(8); Lys,  
3.4(3).

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Compound 31:AVSEHOLLHD KGCSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:45)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Cys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 10 Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:45)

Physical Data: m.p. 140-160°C  $[\alpha]_D^{25}$  -44.48 (c 0.25, H<sub>2</sub>O)FAB(C<sub>172</sub>H<sub>293</sub>N<sub>55</sub>O<sub>51</sub>S<sub>1</sub>): [M+H]<sup>+</sup> 3979

15 AAA: Asx+Cys, 3.0(2+1); Glx, 5.6(6); Ser, 1.7(2); His, 3.0(3);  
 Gly, 1.0(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 2.5(3); Val,  
 1.0(1); Ile, 0.9(1); Leu, 7.5(8); Lys, 3.3(3).

Compound 32:

AVSEHOLLHD KGXSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:46)  
(X = Cys(CH<sub>2</sub>CONH(CH<sub>2</sub>)<sub>2</sub>NH(biotinyl)))

25 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Xaa Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 30 Leu His Thr Ala NH<sub>2</sub>, (Xaa = Cys(CH<sub>2</sub>CONH(CH<sub>2</sub>)<sub>2</sub>NH(biotinyl))), (SEQ  
 ID NO:46)

Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  (not determined)FAB(C<sub>186</sub>H<sub>316</sub>N<sub>59</sub>O<sub>54</sub>S<sub>2</sub>): [M+H]<sup>+</sup> 4306.6

35 AAA: Asx, 2.2(2); Glx, 6.1(6); Ser, 1.8(2); His, 3.8(3); Gly,  
 1.0(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.1(3); Val,  
 1.1(1); Ile, 0.9(1); Leu, 8.3(3); Lys, 3.3(3).

Compound 33:

AVSEHOLLHD KGXSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:47)  
(X = Lys(7-dimethylamino-2-oxo-2H-1-benzopyran-4-acetyl))

45  
 50 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Xaa Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 55 Leu His Thr Ala NH<sub>2</sub>, (Xaa = Lys(7-dimethylamino-2-oxo-2H-1-

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benxopyran-4-acetyl), (SEQ ID NO:47)

Physical Data: m.p. 135-205°C  $[\alpha]_D^{25}$  -26.92 (c 0.104, 50% aq. HOAc)

FAB(C<sub>188</sub>H<sub>311</sub>N<sub>57</sub>O<sub>54</sub>): [M+H]<sup>+</sup> 4233  
 AAA: Asx, 1.9(2); Glx, 6.3(6); Ser, 1.7(2); His, 3.2(3); Gly, 1.0(1); Thr, 1.1(1); Ala, 2.0(2); Arg, 3.2(3); Val, 1.1(1); Ile, 0.9(1); Leu, 8.2(8); Lys, 4.5(4).

Compound 34:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTAG-OH (SEQ ID NO:48)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Ala Gly OH (SEQ ID NO:48)  
 35

Physical Data: m.p. 92.1-146.6°C  $[\alpha]_D^{25}$  -40.76 (c 0.34, H<sub>2</sub>O)  
 FAB(C<sub>177</sub>H<sub>302</sub>N<sub>56</sub>O<sub>53</sub>): [M+H]<sup>+</sup> 4062.0  
 AAA: Asx, 2.0(2); Glx, 5.7(6); Ser, 1.8(2); His, 3.0(3); Gly, 2.2(2); Thr, 0.9(1); Ala, 1.9(2); Arg, 2.8(3); Val, 1.2(1); Ile, 0.9(1); Leu, 7.5(8); Lys, 4.2(4).

Compound 35:

AVSX<sub>1</sub>HOLLHX<sub>2</sub> KGKSIOX<sub>2</sub>LRR RX<sub>1</sub>LLX<sub>1</sub>KLLX<sub>1</sub>K LHA-OH (SEQ ID NO:49)  
 (X<sub>1</sub> = Glu(OCH<sub>3</sub>); X<sub>2</sub> = Asp(OCH<sub>3</sub>))

Ala Val Ser Xaa<sub>1</sub> His Gln Leu Leu His Xaa<sub>2</sub> Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Xaa<sub>2</sub> Leu Arg Arg Arg Xaa<sub>1</sub> Leu Leu Xaa<sub>1</sub> Lys Leu Leu Xaa<sub>1</sub> Lys  
 20 25 30

Leu His Ala OH (Xaa<sub>1</sub> = Glu(OCH<sub>3</sub>); Xaa<sub>2</sub> = Asp(OCH<sub>3</sub>)) (SEQ ID NO:49)

Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  -21.96 (c 0.132, H<sub>2</sub>O)

FAB(C<sub>181</sub>H<sub>311</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4089.0  
 AAA: Asx, 2.1(2); Glx, 6.3(6); Ser, 1.8(2); His, 3.3(3); Gly, 1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.1(3); Val, 1.1(1); Ile, 0.9(1); Leu, 8.0(8); Lys, 4.2(4).

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Compound 36:

AVSX<sub>1</sub>HOLLHX<sub>2</sub> KGKSIOX<sub>3</sub>LRR RX<sub>1</sub>LLX<sub>2</sub>KLLX<sub>3</sub>K LHA-OCH<sub>3</sub> (SEQ ID NO:50)  
(X<sub>1</sub> = Glu(OCH<sub>3</sub>); X<sub>2</sub> = Asp(OCH<sub>3</sub>))

5 Ala Val Ser Xaa<sub>1</sub> His Gln Leu Leu His Xaa<sub>2</sub> Lys Gly Lys Ser Ile  
 1 5 10 15  
 10 Gln Xaa<sub>2</sub> Leu Arg Arg Arg Xaa<sub>1</sub> Leu Leu Xaa<sub>1</sub> Lys Leu Leu Xaa<sub>1</sub> Lys  
 20 25 30

Leu His Ala OCH<sub>3</sub> (Xaa<sub>1</sub> = Glu(OCH<sub>3</sub>); Xaa<sub>2</sub> = Asp(OCH<sub>3</sub>)) (SEQ ID NO:50)

15 Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  -46.80 (c 0.07, H<sub>2</sub>O)  
 FAB(C<sub>182</sub>H<sub>313</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4103  
 20 AAA: Asx, 2.1(2); Glx, 6.2(6); Ser, 1.4(2); His, 3.0(3); Gly, 1.1(1); Thr, 1.1(1); Ala, 1.7(2); Arg, 3.2(3); Val, 0.6(1); Ile, 0.9(1); Leu, 8.0(8); Lys, 4.1(4).

Compound 38:

25 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTAP-OH (SEQ ID NO:52)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 30 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Ala Pro OH (SEQ ID NO:52)  
 35 35

40 Physical Data: m.p. 152.1-186.5°C  $[\alpha]_D^{25}$  -55.91 (c 0.33, H<sub>2</sub>O)  
 FAB(C<sub>180</sub>H<sub>306</sub>N<sub>56</sub>O<sub>53</sub>): [M+H]<sup>+</sup> 4102.6  
 AAA: Asx, 2.0(2); Glx, 5.6(6); Ser, 1.7(2); His, 2.9(3); Gly, 1.1(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 2.9(3); Val, 1.2(1); Ile, 1.0(1); Leu, 7.7(8); Lys, 4.3(4).

Compound 39:

45 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTP-OH (SEQ ID NO:53)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 50 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

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Leu His Thr Pro OH (SEQ ID NO:53)

Physical Data: m.p. 120-148.2°C  $[\alpha]_D^{25}$  -52.78 (c 0.47, H<sub>2</sub>O)

FAB(C<sub>177</sub>H<sub>301</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4031.0

AAA: Asx, 2.0(2); Glx, 5.5(6); Ser, 1.8(2); His, 2.9(3); Gly, 1.0(1); Thr, 1.0(1); Ala, 0.9(1); Arg, 2.9(3); Val, 1.2(1); Ile, 0.9(1); Leu, 7.5(8); Lys, 3.6(3); Pro, 0.9(1).

Compound 40:AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTP-NH<sub>2</sub> (SEQ ID NO:54)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Pro NH<sub>2</sub> (SEQ ID NO:54)

Physical Data: m.p. 133.9-155.1°C  $[\alpha]_D^{25}$  -54.22 (c 0.37, H<sub>2</sub>O)

FAB(C<sub>177</sub>H<sub>302</sub>N<sub>56</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4030.7

AAA: Asx, 2.0(2); Glx, 5.6(6); Ser, 1.9(2); His, 2.9(3); Gly, 1.1(1); Thr, 0.9(1); Ala, 0.9(1); Arg, 2.8(3); Val, 1.2(1); Ile, 1.1(1); Leu, 7.8(8); Lys, 4.2(4); Pro, 0.9(1).

Compound 41:AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHP-NH<sub>2</sub> (SEQ ID NO:55)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Pro NH<sub>2</sub> (SEQ ID NO:55)

Physical Data: m.p. 142.8-166.1°C  $[\alpha]_D^{25}$  -53.80 (c 0.38, H<sub>2</sub>O)

FAB(C<sub>173</sub>H<sub>295</sub>N<sub>55</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 3929

AAA: Asx, 2.0(2); Glx, 5.7(6); Ser, 1.8(2); His, 3.0(3); Gly, 1.1(1); Ala, 0.9(1); Arg, 2.8(3); Val, 1.2(1); Ile, 0.9(1); Leu, 7.4(8); Lys, 4.4(4); Pro, 0.9(1).

Compound 42:AVSEHOLLHD KGKSIODLRR RELLEKLLEK LP-NH<sub>2</sub> (SEQ ID NO:56)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

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Gln Asp Leu Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu Pro NH<sub>2</sub> (SEQ ID NO:56)

5 Physical Data: m.p. 161.0-177.0°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -61.97 (c 0.19, H<sub>2</sub>O)  
FAB(C<sub>167</sub>H<sub>288</sub>N<sub>52</sub>O<sub>48</sub>): [M+H]<sup>+</sup> 3792.0  
AAA: Asx, 2.2(2); Glx, 5.9(6); Ser, 1.9(2); His, 2.1(2); Gly,  
1.1(1); Ala, 1.0(1); Arg, 3.0(3); Val, 1.1(1); Ile,  
10 1.0(1); Leu, 7.9(8); Lys, 4.3(4); Pro, 0.9(1).

Compound 43:

15 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTRSAW-OH (SEQ ID NO:57)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

20 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Arg Ser Ala Trp OH (SEQ ID NO:57)  
35

25 Physical Data: m.p. 181-202°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -45.14 (c 0.19, H<sub>2</sub>O)  
FAB(C<sub>195</sub>H<sub>326</sub>N<sub>62</sub>O<sub>56</sub>): [M+H]<sup>+</sup> 4435.2  
AAA: Asx, 2.0(2); Glx, 5.8(6); Ser, 2.8(3); His, 2.8(3); Gly,  
1.1(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 3.7(4); Ile,  
30 0.9(1); Leu, 7.5(8); Lys, 4.3(4); Trp, 0.9(1).

Compound 44:

35 AVSEHOLLHD RGRSIODLRR RELLERLER LHTAGRRTSAW-OH (SEQ ID NO:58)

40 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

45 Leu His Thr Arg Gly Arg Arg Thr Arg Ser Ala Trp OH (SEQ ID NO:58)  
35 40

50 Physical Data: m.p. 130-132.2°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -46.66 (c 0.195, H<sub>2</sub>O)  
FAB(C<sub>216</sub>H<sub>365</sub>N<sub>81</sub>O<sub>62</sub>): [M+H]<sup>+</sup> 5088.8  
AAA: Asx, 2.2(2); Glx, 6.0(6); Ser, 2.7(3); His, 3.0(3); Gly,  
2.2(2); Thr, 2.1(2); Ala, 3.0(3); Arg, 10.5(10); Val,  
0.9(1); Ile, 1.0(1); Leu, 8.2(8); Trp, 1.0(1).

55

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Compound 45:

AVSEHOLLHD RGRSIODLRR RELLERLLER LHTAGRRTSAW-NH<sub>2</sub> (SEQ ID NO:59)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
1 5 10 15

10 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

Leu His Thr Arg Gly Arg Arg Thr Arg Ser Ala Trp NH<sub>2</sub> (SEQ ID NO:59)  
35 40

15 Physical Data: m.p. 158-174°C  $[\alpha]_D^{25}$  -43.57 (c 0.53, H<sub>2</sub>O)  
FAB(C<sub>216</sub>H<sub>366</sub>N<sub>82</sub>O<sub>61</sub>): [M+H]<sup>+</sup> 5087.4  
20 AAA: Asx, 1.9(2); Glx, 5.6(6); Ser, 2.6(3); His, 3.3(3); Gly, 2.1(2); Thr, 2.0(2); Ala, 2.9(3); Arg, 10.1(10); Val, 0.9(1); Ile, 1.0(1); Leu, 8.3(8); Trp 1.1(1).

Compound 46:

25 AVSEHOLLHD RGXSIODLRR RELLERLLER LHTAGRRTSAW-OH (SEQ ID NO:60)

(X = Lys(dihydrocinnamoyl))

30 Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Xaa Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
20 25 30

35 Leu His Thr Arg Gly Arg Arg Thr Arg Ser Ala Trp OH (Xaa =  
35 40

Lys(dihydrocinnamoyl), (SEQ ID NO:60)

40 Physical Data: m.p. 165.4-175.2°C  $[\alpha]_D^{25}$  -40.43 (c 0.20, H<sub>2</sub>O)  
FAB(C<sub>225</sub>H<sub>374</sub>N<sub>80</sub>O<sub>62</sub>): [M+H]<sup>+</sup> 5191  
45 AAA: Asx, 2.1(2); Glx, 6.3(6); Ser, 2.8(3); His, 3.2(3); Gly, 2.1(2); Thr, 2.0(2); Ala, 3.2(3); Arg, 9.9(9); Val, 1.0(1); Ile, 0.9(1); Leu, 8.6(8); Lys, 1.1(1); Trp, 1.1(1).

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Compound 47:

AVSEIOFXHN LGKHLSSXTR SAWLRKKLOD VHNY-NH<sub>2</sub> (SEQ ID NO:61)  
(X = norleucine)

Ala Val Ser Glu Ile Gln Phe Nle His Asn Leu Gly Lys His Leu  
 1 5 10 15

Ser Ser Nle Thr Arg Ser Ala Trp Leu Arg Lys Lys Leu Gln Asp  
 20 25 30

Val His Asn Tyr NH<sub>2</sub> (SEQ ID NO:61)

Physical Data: m.p. 140-160°C  $[\alpha]_D^{25}$  -56.88 (c 0.16, H<sub>2</sub>O)  
 FAB(C<sub>180</sub>H<sub>287</sub>N<sub>55</sub>O<sub>58</sub>): [M+H]<sup>+</sup> 3989.8  
 AAA: Asx, 3.0(3); Glx, 2.9(3); Ser, 3.7(4); His, 2.8(3); Gly,  
 1.1(1); Thr, 0.9(1); Ala, 1.9(2); Arg, 2.0(2); Tyr,  
 1.0(1); Val, 1.7(2); Phe, 0.9(1); Ile, 0.9(1); Leu+Nle  
 5.8(2+4); Lys, 3.4(3); Trp, 1.1(1).

Compound 48:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTMA-NH<sub>2</sub> (SEQ ID NO:62)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Met Ala NH<sub>2</sub> (SEQ ID NO:62)  
 35

Physical Data: m.p. 140-210°C  $[\alpha]_D^{25}$  -47.75 (c 0.178, H<sub>2</sub>O)  
 FAB(C<sub>180</sub>H<sub>309</sub>N<sub>57</sub>O<sub>52</sub>S<sub>1</sub>): [M+H]<sup>+</sup> 4135.0  
 AAA: Asx, 2.3(2); Glx, 6.6(6); Ser, 1.4(2); His, 3.2(3); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.1(3); Val,  
 0.9(1); Met, 1.1(1); Ile, 1.0(1); Leu, 8.8(8); Lys,  
 4.4(4).

Compound 50:

AVSEHOLLHD KGKSIODLRR RFFLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:64)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Phe Phe Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:64)  
 35



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Physical Data: m.p. 136.5-156.8°C  $[\alpha]_D^{25}$  -49.89 (c 0.24, H<sub>2</sub>O)  
 FAB(C<sub>182</sub>H<sub>300</sub>N<sub>56</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4056.0  
 AAA: Asx, 2.2(2); Glx, 5.0(5); Ser, 1.9(2); His, 3.3(3); Gly,  
 1.0(1); Thr, 1.0(1); Ala, 2.1(2); Arg, 3.1(3); Val,  
 1.0(1); Phe, 2.0(2); Ile, 0.9(1); Leu, 7.2(7); Lys,  
 3.5(4).

Compound 51:

AVSEHOLLHD KGKSIODLRR RELHKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:65)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu His Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:65)

Physical Data: m.p. 80.7-141.0°C  $[\alpha]_D^{25}$  -55.38 (c 0.23, H<sub>2</sub>O)  
 FAB(C<sub>176</sub>H<sub>300</sub>N<sub>58</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4012.8  
 AAA: Asx, 2.2(2); Glx, 4.9(5); Ser, 1.8(2); His, 4.3(4); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.1(3); Val,  
 1.1(1); Ile, 1.0(1); Leu, 8.1(8); Lys, 3.9(4).

Compound 52:

AVSEHOLLHD KGKSIODLRR RELLEHLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:66)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu His Leu Leu Glu Lys  
 20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:66)

Physical Data: m.p. 134.3-157.9°C  $[\alpha]_D^{25}$  -50.72 (c 0.45, H<sub>2</sub>O)  
 FAB(C<sub>175</sub>H<sub>295</sub>N<sub>57</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4012.8  
 AAA: Asx, 2.1(2); Glx, 5.9(6); Ser, 1.8(2); His, 4.2(4); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.0(2); Arg, 3.0(3); Val,  
 1.1(1); Ile, 0.9(1); Leu, 8.1(8); Lys, 3.1(3).

Compound 53:

AVSEHOLLHD KGKSIODLRR RELLEKLI AK LHTA-NH<sub>2</sub> (SEQ ID NO:67)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

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Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Ile Ala Lys  
20 25 30

Leu His Thr Ala NH<sub>2</sub> (SEQ ID NO:67)

5 Physical Data: m.p. 142.7-159.8°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -54.01 (c 0.21, H<sub>2</sub>O)  
FAB(C<sub>173</sub>H<sub>298</sub>N<sub>56</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 3946.0  
AAA: Asx, 2.2(2); Glx, 4.9(5); Ser, 1.8(2); His, 3.1(3); Gly,  
1.1(1); Thr, 1.0(1); Ala, 3.1(3); Arg, 3.1(3); Val,  
10 1.0(1); Ile, 1.9(2); Leu, 7.0(7); Lys, 4.3(4).

Compound 54:

15 AVSEHOLLHD KGKSIODLRR RELLEKLLEE IHTA-NH<sub>2</sub> (SEQ ID NO:68)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15  
20 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Glu  
20 25 30

Ile His Thr Ala NH<sub>2</sub> (SEQ ID NO:68)

25 Physical Data: m.p. 138-185°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -50.17 (c 0.14, H<sub>2</sub>O)  
FAB(C<sub>174</sub>H<sub>295</sub>N<sub>55</sub>O<sub>53</sub>): [M+H]<sup>+</sup> 4005  
AAA: Asx, 2.2(2); Glx, 7.1(7); Ser, 1.7(2); His, 2.8(3); Gly,  
1.0(1); Thr, 1.0(1); Ala, 2.1(2); Arg, 3.1(3); Val,  
30 1.1(1); Ile, 1.7(2); Leu, 7.1(7); Lys, 2.7(3).

Compound 58:

35 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTRSAW-NH<sub>2</sub> (SEQ ID NO:72)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15  
40 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Arg Ser Ala Trp NH<sub>2</sub> (SEQ ID NO:72)  
35

45 Physical Data: m.p. 158-163°C [ $\alpha$ ]<sub>D</sub><sup>25</sup> -46.06 (c 0.17, H<sub>2</sub>O)  
FAB(C<sub>195</sub>H<sub>327</sub>N<sub>63</sub>O<sub>55</sub>): [M+H]<sup>+</sup> 4434.8  
AAA: Asx, 2.0(2); Glx, 5.5(6); Ser, 2.7(3); His, 3.1(3); Gly,  
50 1.0(1); Ala, 1.8(2); Arg, 4.0(4); Thr, 0.9(1); Val,  
0.9(1); Ile, 0.9(1); Leu, 7.5(8); Lys, 3.9(4); Trp,  
1.0(1).

55

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Compound 59:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTRSAX-OH (SEQ ID NO:73)  
(X = Nal(2) = 3-(2-naphthyl)-L-alanine)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

10 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr Arg Ser Ala 3-(2-naphthyl)-L-alanine-OH (SEQ ID  
 NO:73)  
 35

15 Physical Data: m.p. 156-162°C  $[\alpha]_D^{25}$  -44.44 (c 0.189, H<sub>2</sub>O)  
 FAB(C<sub>197</sub>H<sub>328</sub>N<sub>62</sub>O<sub>55</sub>): [M+H]<sup>+</sup> 4445.6  
 AAA: Asx, 2.1(2); Glx, 5.5(6); Ser, 2.8(3); His, 2.9(3); Gly,  
 1.0(1); Ala, 2.0(2); Arg, 4.0(4); Thr, 0.9(1); Val,  
 20 1.0(1); Ile, 0.9(1); Leu, 7.5(8); Lys, 4.2(4); Nal,  
 1.1(1).

Compound 60:

25 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTASAW-OH (SEQ ID NO:74)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

30 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

35 Leu His Thr Ala Ser Ala Trp OH (SEQ ID NO:74)  
 35

40 Physical Data: m.p. 159-164°C  $[\alpha]_D^{25}$  -50.94 (c 0.29, H<sub>2</sub>O)  
 FAB(C<sub>192</sub>H<sub>320</sub>N<sub>60</sub>O<sub>55</sub>): [M+H]<sup>+</sup> 4349.0  
 AAA: Asx, 2.0(2); Glx, 5.6(6); Ser, 2.7(3); His, 3.2(3); Gly,  
 1.0(1); Ala, 3.1(3); Arg, 2.8(3); Thr, 1.0(1); Val,  
 1.1(1); Ile, 0.9(1); Leu, 7.6(8); Lys, 4.0(4); Trp,  
 1.0(1).

Compound 61:

45 AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTAEIRA-OH (SEQ ID NO:75)

50 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

55

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Leu His Thr Ala Glu Ile Arg Ala OH (SEQ ID NO:75)  
35

Physical Data: m.p. 155-210°C  $[\alpha]_D^{25}$  -46.15 (c 0.12, H<sub>2</sub>O)  
FAB(C<sub>195</sub>H<sub>334</sub>N<sub>62</sub>O<sub>58</sub>): [M+H]<sup>+</sup> 4475.8  
AAA: Asx, 2.2(2); Glx, 6.9(7); Ser, 1.7(2); His, 3.2(3); Gly,  
1.1(1); Ala, 3.1(3); Arg, 4.0(4); Thr, 0.9(1); Val,  
1.1(1); Ile, 1.9(2); Leu, 8.1(8); Lys, 4.1(4).

Compound 62:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHAEIR-OH (SEQ ID NO:76)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Ala Glu Ile Arg OH (SEQ ID NO:76)  
35

Physical Data: m.p. 186-218°C  $[\alpha]_D^{25}$  -52.73 (c 0.265, H<sub>2</sub>O)  
FAB(C<sub>192</sub>H<sub>329</sub>N<sub>61</sub>O<sub>57</sub>): [M+H]<sup>+</sup> 4404.4  
AAA: Asx, 2.0(2); Glx, 6.6(7); Ser, 1.9(2); His, 3.4(3); Gly,  
1.1(1); Ala, 2.0(2); Arg, 3.8(4); Thr, 1.0(1); Val,  
1.1(1); Ile, 1.7(2); Leu, 7.9(8); Lys, 4.0(4).

Compound 63:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHAEI-OH (SEQ ID NO:77)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr Ala Glu Ile OH (SEQ ID NO:77)  
35

Physical Data: m.p. 169-205°C  $[\alpha]_D^{25}$  -50.78 (c 0.51, H<sub>2</sub>O)  
FAB(C<sub>186</sub>H<sub>317</sub>N<sub>57</sub>O<sub>56</sub>): [M+H]<sup>+</sup> 4248.0  
AAA: Asx, 2.2(2); Glx, 6.8(7); Ser, 1.8(2); His, 3.3(3); Gly,  
1.0(1); Ala, 2.0(2); Arg, 3.0(3); Thr, 1.0(1); Val,  
1.0(1); Ile, 1.8(2); Leu, 7.8(8); Lys, 3.6(4).

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Compound 64:AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTAE-OH (SEQ ID NO:78)

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 10 Leu His Thr Ala Glu OH (SEQ ID NO:78)  
 35

Physical Data: m.p. 199-205°C  $[\alpha]_D^{25}$  -52.47 (c 0.41, H<sub>2</sub>O)  
 15 FAB(C<sub>180</sub>H<sub>306</sub>N<sub>56</sub>O<sub>55</sub>): [M+H]<sup>+</sup> 4135.0  
 AAA: Asx, 2.0(2); Glx, 6.6(7); Ser, 1.9(2); His, 3.3(3); Gly,  
 1.1(1); Ala, 2.0(2); Arg, 2.9(3); Thr, 1.0(1); Val,  
 1.1(1); Ile, 1.0(1); Leu, 8.2(8); Lys, 3.8(4).

20

Compound 66:SEHOLLHD KGKSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:80)

25 Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp  
 1 5 10 15  
 30 Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His  
 20 25 30  
 Thr Ala NH<sub>2</sub> (SEQ ID NO:80)

35 Physical Data: m.p. 134.2°C  $[\alpha]_D^{25}$  -48.12 (c 0.36, H<sub>2</sub>O)  
 FAB(C<sub>167</sub>H<sub>286</sub>N<sub>54</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 3834.4  
 AAA: Asx, 2.0(2); Glx, 5.7(6); Ser, 1.7(2); His, 2.9(3); Gly,  
 1.0(1); Thr, 0.9(1); Ala, 1.0(1); Arg, 2.8(3); Ile,  
 0.9(1); Leu, 7.4(8); Lys, 4.3(4).

40

Compound 67:LLHD KGKSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:81)

45 Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg Arg  
 1 5 10 15  
 50 Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH<sub>2</sub> (SEQ ID  
 NO:81)  
 20 25

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Physical Data: m.p. 128.5-184.5°C  $[\alpha]_D^{25}$  -6.53 (c 0.69, MeOH)  
 FAB(C<sub>148</sub>H<sub>259</sub>N<sub>47</sub>O<sub>41</sub>): [M+H]<sup>+</sup> 3353  
 AAA: Asx, 2.0(2); Glx, 4.1(4); Ser, 0.9(1); His, 2.1(2); Gly,  
 1.0(1); Thr, 0.9(1); Ala, 1.0(1); Arg, 3.0(3); Ile,  
 1.0(1); Leu, 8.1(8); Lys, 4.2(4).

Compound 68:LHD KGKSIODLRR RELLEKLLEK LHTA-NH<sub>2</sub> (SEQ ID NO:82)

Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg Arg Glu  
 1 5 10 15

Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH<sub>2</sub> (SEQ ID  
 NO:82) 20 25

Physical Data: m.p. 165-210°C  $[\alpha]_D^{25}$  -36.05 (c 0.12, H<sub>2</sub>O)  
 FAB(C<sub>142</sub>H<sub>248</sub>N<sub>46</sub>O<sub>40</sub>): [M+H]<sup>+</sup> 3239.0  
 AAA: Asx, 2.0(2); Glx, 3.9(4); Ser, 0.9(1); His, 1.9(2); Gly,  
 1.0(1); Thr, 1.0(1); Ala, 1.0(1); Arg, 2.9(3); Ile,  
 0.9(1); Leu, 6.8(7); Lys, 4.2(4).

Compound 69:SEHQLLHD RGRSIODLRR RELLERLLER LHAGRRTRSAW-OH (SEQ ID NO:83)

Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile Gln Asp  
 1 5 10 15

Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg Leu His  
 20 25 30

Leu His Arg Gly Arg Arg Thr Arg Ser Ala Trp OH (SEQ ID NO:83)  
 35 40

Physical Data: m.p. 150-210°C  $[\alpha]_D^{25}$  -18.0 (c 0.64, H<sub>2</sub>O)  
 FAB(C<sub>208</sub>H<sub>351</sub>N<sub>79</sub>O<sub>60</sub>): [M+H]<sup>+</sup> 4918.6  
 AAA: Asx, 2.2(2); Glx, 6.2(6); Ser, 2.8(3); His, 3.1(3); Gly,  
 2.2(2); Thr, 2.2(2); Ala, 2.2(2); Arg, 10.4(10); Ile,  
 1.0(1); Leu, 8.0(8); Trp, 1.1(1).

Compound 70:LLHD RGRSIODLRR RELLERLLER LHAGRRTRSAW-OH (SEQ ID NO:84)

Leu Leu His Asp Arg Gly Arg Ser Ile Gln Asp Leu Arg Arg Arg  
 1 5 10 15



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**EXAMPLE II**

[Met<sup>34</sup>, Ala<sup>35</sup>] Compound 1, AVSEHQLLHDKGKSIQDLRRRELLEK-LLEKLHTMA-NH<sub>2</sub>, (SEQ ID NO:25), was prepared and purified following the procedures of Example I. This polypeptide was converted to the homoserine lactone as follows. The purified peptide (160 mgs) was dissolved in 44% formic acid (4 mL). This solution was combined with a premixed solution of cyanogen bromide (700 mgs) and phenol (1.6 mgs) in 44% formic acid (4 mL) at 0°C. The solution was stirred at 0°C for 2 hr and at room temperature for 2 hrs. The formation of the product was monitored by HPLC (Vydac® C-18, 300 Å, 4.6 x 250 mm, flow of 1.2 mL/min, gradient 25-45% acetonitrile in 0.1% TFA over 10 min). The reaction was complete within 4 hr. Half of the sample was concentrated and purified by preparative RP-HPLC (Vydac® C-18, gradient 25-45% acetonitrile in 0.1% TFA). The homoserine lactone peptide fractions were pooled and lyophilized to give 28 mgs of white solid of >95% purity, Compound 4.

Compound 4AVSEHQLLHDKGKSIQDLRRRELLEKLLEKLHTX (X=hSerlac, SEQ ID NO:9)

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1				5					10					15
Gln	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys
			20						25					30

Leu His Thr hSerlac (SEQ ID NO:9)

Physical Data:

m.p. 138-142°C

[α]<sub>D</sub><sup>25</sup> -50.66° (c 0.1, H<sub>2</sub>O)FAB (C<sub>176</sub>H<sub>299</sub>N<sub>55</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4017.61

AAA: Asp, 2.1(2); Glu, 6.1(6); Ser, 1.8(2); His, 3.0(3); Thi,  
 1.1(1); Ala, 1.1(1); Arg, 2.7(3); Val, 1.0(1); Ile,  
 1.0(1); Leu, 8.2(8); Lys, 3.8(4); Gly 1.09(1); hSer,  
 1.09(1).

Similarly, Compound 65 was prepared in accordance with this procedure.



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Compound 65:

AVSEIQFX,HN KGKHLSSX,ER VEWLRKKLOD VHNX, (SEQ ID NO:79)  
(X<sub>1</sub> = L-norleucine; X<sub>2</sub> = homoserine lactone)

5 Ala Val Ser Glu Ile Gln Phe Nle His Asn Lys Gly Lys His Leu  
 1 5 10 15  
 10 Ser Ser Nle Glu Arg Val Glu Trp Leu Arg Lys Lys Leu Gln Asp  
 20 25 30  
 Val His Asn hSerlac (SEQ ID NO:79)

15 Physical Data: m.p. 166-176°C  $[\alpha]_D^{25}$  -52.22 (c 0.25, H<sub>2</sub>O)  
 FAB (C<sub>180</sub>H<sub>288</sub>N<sub>54</sub>O<sub>50</sub>): [M+H]<sup>+</sup> 4008.6  
 20 AAA: Asx, 3.1(3); Glx, 4.8(5); Ser, 2.9(3); His, 2.9(3); Gly,  
 1.1(1); Ala, 1.1(1); Arg, 2.0(2); Val, 2.7(3); Phe,  
 1.0(1); Ile, 1.0(1); Leu + Nle 5.9 (4 + 2); Lys, 2.8(3).

EXAMPLE III

25 To prepare the homoserine amide, the crude hSerlactone  
 analog, Compound 4, was concentrated and treated with 25 mL  
 saturated NH<sub>3</sub> in methanol. The solution was stirred at 0°C for  
 2 hr and at room temperature for 16 hr. The reaction was  
 monitored by HPLC (Vydac® C-18, 300 Å, 4.6 x 250 mm, flow of  
 1.2 mL/min, gradient 20-45% acetonitrile in 0.1% TFA) and was  
 30 complete within 18 hr. The solution was concentrated and  
 purified by preparative RP-HPLC (Vydac® C-18, gradient of 25-  
 45% acetonitrile in 0.1% TFA). The homoserine amide peptide  
 fractions were pooled and lyophilized to give 30 mgs of white  
 solid of >98% purity, Compound 3.

Compound 3

AVSEHOLLHDKGKSIODLRRELLEKLLEKLHTX-NH<sub>2</sub> (X=hSer, SEQ ID NO:8)

40 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 45 Leu His Thr hSer NH<sub>2</sub> (SEQ ID NO:8).

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Physical Data:

m.p. 138-142°C  $[\alpha]_D^{25}$  -45.97° (c 0.25, H<sub>2</sub>O)  
 FAB (C<sub>176</sub>H<sub>302</sub>N<sub>56</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4033.9  
 AAA: Asp, 2.1(2); Glu, 6.1(6); Ser, 1.6(2); His, 2.8(3); Gly,  
 0.97(1); hSer, 0.97(1); Thi, 1.0(1); Ala, 1.0(1); Arg,  
 2.9(3); Val, 1.0(1); Ile, 1.0(1); Leu, 7.6(8); Lys,  
 3.9(4).

Similarly, Compounds 22, 23 and 28 were prepared  
 following this procedure.

Compound 22:

AVSEIOFLHN LGKHLSSLRR RELLEKLLEK LHNX-NH<sub>2</sub> (SEQ ID NO:36)  
(X = homoserine)

Ala Val Ser Glu Ile Gln Phe Leu His Asn Leu Gly Lys His Leu  
 1 5 10 15

Ser Ser Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Asn hSer NH<sub>2</sub> (SEQ ID NO:36)

Physical Data: m.p. 69.4-128°C  $[\alpha]_D^{25}$  -43.93 (c 0.15, H<sub>2</sub>O)  
 FAB (C<sub>179</sub>H<sub>302</sub>N<sub>56</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4022.9  
 AAA: Asx, 2.0(2); Glx, 4.9(5); Ser, 2.6(3); His, 2.8(3); Gly,  
 1.0(1); Ala, 1.0(1); Arg, 3.0(3); Val, 1.0(1); Phe,  
 1.0(1); Ile, 0.9(1); Leu, 8.8(9); Lys, 3.4(3);

Compound 23:

AVSEIOFLHN KGKHLSSLRR RELLEKLLEK LHNX-NH<sub>2</sub> (SEQ ID NO:37)  
(X = homoserine)

Ala Val Ser Glu Ile Gln Phe Leu His Asn Lys Gly Lys His Leu  
 1 5 10 15

Ser Ser Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Asn hSer NH<sub>2</sub> (SEQ ID NO:37)

Physical Data: m.p. 87.1-142.1°C  $[\alpha]_D^{25}$  -52.14 (c 0.41, H<sub>2</sub>O)  
 FAB (C<sub>179</sub>H<sub>303</sub>N<sub>57</sub>O<sub>49</sub>): [M+H]<sup>+</sup> 4038  
 AAA: Asx, 2.1(2); Glx, 4.9(5); Ser, 2.7(3); His, 2.8(3); Gly,  
 1.0(1); hSer, 1.0(1); Ala, 1.0(1); Arg, 3.0(3); Val,  
 1.1(1); Phe, 0.9(1); Ile, 0.9(1); Leu, 7.9(8); Lys,  
 3.7(4).

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Compound 28:

AVSEHOLLHD KGKSIODLRR RELLERLLER LHTAGRRX-NH<sub>2</sub> (SEQ ID NO:42)  
(X = homoserine)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg  
 20 25 30

Leu His Thr Ala Gly Arg Arg hSer NH<sub>2</sub> (SEQ ID NO:42)  
 35

Physical Data: m.p. 80°C  $[\alpha]_D^{25}$  -48.64 (c 0.09, H<sub>2</sub>O)  
 FAB(C<sub>193</sub>H<sub>334</sub>N<sub>70</sub>O<sub>56</sub>): [M+H]<sup>+</sup> 4530.0  
 AAA: Asx, 2.2(2); Glx, 6.1(6); Ser, 1.7(2); His, 3.0(3); Gly,  
 1.9(2); hSer, 1.0(1); Thr, 1.0(1); Ala, 2.1(2); Arg,  
 7.2(7); Val, 0.8(1); Ile, 1.0(1); Leu, 8.4(8); Lys,  
 2.1(2).

The homoserine alkylamides were similarly prepared from  
 the homoserine lactone by dissolving it in DMF containing an  
 excess of the corresponding alkylamine. After stirring at  
 room temperature for several days (the reaction was monitored  
 by analytical HPLC) the mixture was evaporated to dryness and  
 the peptide purified by preparative HPLC. Representative  
 homoserine alkylamides are Compounds 55 and 56.

Compound 55:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTX-NHCH<sub>2</sub>CH<sub>3</sub> (SEQ ID NO:69)  
(X = homoserine)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30

Leu His Thr hSer NHCH<sub>2</sub>CH<sub>3</sub> (SEQ ID NO:69)

Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  (not determined)  
 FAB(C<sub>178</sub>H<sub>306</sub>N<sub>56</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4063.0  
 AAA: Asx, 2.1(2); Glx, 5.8(6); Ser, 1.7(2); His, 3.1(3);  
 Gly, 0.9(1); Thr, 1.0(1); Ala, 0.9(1); Arg, 3.0(3); Val,  
 1.1(1); Ile, 1.0(1); Leu, 8.4(8); Lys, 3.7(4); hSer,  
 0.9(1).

Compound 56:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTX-NHCH<sub>2</sub>CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> (SEQ ID NO:70)

(X = homoserine)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

10 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr hSer NHCH<sub>2</sub>CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub> (SEQ ID NO:70)

15 Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  (not determined)  
FAB(C<sub>184</sub>H<sub>310</sub>N<sub>56</sub>O<sub>52</sub>): [M+H]<sup>+</sup> 4138.8  
AAA: Asx, 2.0(2); Glx, 5.9(6); Ser, 1.7(2); His, 2.9(3); Gly,  
0.9(1); Thr, 1.0(1); Ala, 0.9(1); Arg, 3.0(3); Val,  
1.0(1); Ile, 0.9(1); Leu, 8.0(8); Lys, 4.1(4); hSer,  
20 0.9(1).

EXAMPLE IV

25 The cesium salt of N<sup>α</sup>-Boc-N<sup>γ</sup>-Fmoc-L-2,4-diaminobutyric  
acid was attached to Merrifield resin (DMF, 50°C, 48hrs) and  
used in a solid-phase synthesis in place of the Boc-Ala resin  
as in Example I. Upon completion of the synthesis the peptide  
was treated with 20% piperidine in DMF at room temperature for  
30 minutes to remove the side chain Fmoc protecting group.  
30 The protected peptide spontaneously cyclized to the lactam  
thereby cleaving itself from the resin. The solution was  
filtered from the resin and evaporated under vacuum to leave  
on oil. The residue was treated with liquid HF as in Example  
I to yield the crude unprotected peptide. The peptide was  
35 treated and purified as in Example I.

Compound 49:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTX (SEQ ID NO:63)  
(X = L-2,4-diaminobutyryl lactam)

40 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

45 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
20 25 30

Leu His Thr L-2,4-diaminobutyryl lactam (SEQ ID NO:63)

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Physical Data: m.p. 161-181°C  $[\alpha]_D^{25}$  -48.38 (c 0.25, H<sub>2</sub>O)  
 FAB(C<sub>176</sub>H<sub>300</sub>N<sub>56</sub>O<sub>51</sub>): [M+H]<sup>+</sup> 4016.8  
 AAA: Asx, 2.1(2); Glx, 6.3(6); Ser, 1.7(2); His, 3.3(3); Gly,  
 1.1(1); Thr, 1.0(1); Ala, 2.1(2); Arg, 2.9(3); Val,  
 0.9(1); Ile, 0.9(1); Leu, 8.0(8); Lys, 3.8(4).

## EXAMPLE V

An aqueous solution of the homoserine lactone analog from  
 Example II was treated with porcine liver esterase (Sigma  
 Chemical Company, St. Louis, MO). The hydrolysis of the  
 lactone to the C-terminal homoserine was monitored by  
 analytical HPLC. When the hydrolysis was judged to be  
 complete the material was purified by preparative HPLC as in  
 Example I.

## Compound 37:

AVSEHOLLHD KGKSIODLRR RELLEKLLEK LHTX-OH (SEQ ID NO:51)  
(X = homoserine)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15  
 Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys  
 20 25 30  
 Leu His Thr hSer OH (SEQ ID NO:51)

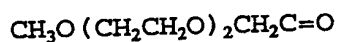
Physical Data: m.p. (not determined)  $[\alpha]_D^{25}$  (not determined)  
 FAB(C<sub>176</sub>H<sub>301</sub>N<sub>55</sub>O<sub>53</sub>): [M+H]<sup>+</sup> 4035.1  
 AAA: Asx, 2.1(2); Glx, 5.9(6); Ser, 2.0(2); His, 3.1(3); Gly,  
 0.8(1); hSer, 0.8(1); Thr, 1.0(1); Ala, 1.0(1); Arg,  
 3.0(3); Val, 1.3(1); Ile, 1.0(1); Leu, 8.1(8); Lys,  
 3.8(4).

## EXAMPLE VI

Following Example I, the protected peptide-resin  
 BocAVS(Bzl)E(OBz)H(Bom)QLLHD(OBzl)R(Ts)GR(Ts)S(Bzl)IQD(OBz)-  
 LR(Ts)R(Ts)E(OBz)LLE(OBzl)R(Ts)LLK(Fmoc)R(Ts)LH(Bom)T(Bzl)A-  
 O-PAM was synthesized on a 0.35 mmol scale. All N<sup>α</sup> groups were  
 protected with t-butoxycarbonyl (Boc); side chain protecting  
 groups were as indicated. After completion of the synthesis,  
 the peptide resin was treated with 50 mL of 20% piperidine in  
 dimethylformamide (DMF) at room temperature for 30 minutes to  
 remove the fluorenylmethoxy-carbonyl (Fmoc) protecting group

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on lysine. The resin was washed successively with DMF, MeOH,  $\text{CH}_2\text{Cl}_2$  and dried to give 1.6 g partially protected peptide. 0.8 g (0.175 mmol) of the partially protected peptide was acylated on lysine with 0.44 g (0.3 mmol) of methoxydi(ethyleneoxy) acetic acid [PEG(2) $\text{CH}_2\text{COOH}$ ] in the presence of 0.16 g (0.3 mmol) benzotriazolyloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBop) and 0.067 g (0.525 mmol) of diisopropylethyl amine (DIEA) in 20 mL DMF at room temperature for 5 hrs. After 5 hrs., the resin was filtered and washed successively with DMF, MeOH and  $\text{CH}_2\text{Cl}_2$ . The acylation step was repeated twice until negative ninhydrin result on the resin was obtained. The final peptide was cleaved from the resin with removal of the side chain protecting groups and purification as in Example I; 100 mgs of Compound 19 were obtained.

Compound 19AVSEHOLLHDRGRSIDIOLRRRELLERLLKRLHTA-OH (SEQ ID NO:18)

Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Arg	Gly	Arg	Ser	Ile
1				5					10					15
Gln	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu		
						20						25		

Lys(COCH<sub>2</sub>PEG2) Arg Leu His Thr Ala OH (SEQ ID NO:18)

30

Physical Data:

m.p. 145-195°C

[ $\alpha$ ]<sub>D</sub><sup>25</sup> -44.60° (c 0.2, H<sub>2</sub>O)FAB (C<sub>183</sub>H<sub>316</sub>N<sub>64</sub>O<sub>54</sub>): [M+H]<sup>+</sup> 4276.2

AAA: Asp, 2.1(2); Glu, 5.0(5); Ser, 1.6(2); His, 2.9(3); Gly, 0.9(1); Thr, 1.9(2); Arg, 7.1(7); Val, 1.1(1); Ile, 1.0(1); Leu, 8.0(8); Lys, 0.9(1).

EXAMPLE VII

Peptide was synthesized, cleaved and purified in the same manner as in Example IV except 2-methoxypoly(ethylene-oxy) acetic acid [PEG(5000) $\text{CH}_2\text{CO}_2\text{H}$ ] was used as the acylating agent. 0.8 g (0.175 mmol) of partially protected peptide yielded 300 mgs of pure Compound 20.

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Compound 20AVSEHOLLHDRGRSIODLRRRELLERLLKRLHTA-OH (SEQ ID NO:19)

Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
 1 5 10 15

Gln Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu  
 20 25

Lys(COCH<sub>2</sub>PEG5000) Arg Leu His Thr Ala OH (SEQ ID NO:19).  
 30

Physical Data:

m.p. 105°C  $[\alpha]_D^{25}$  -22.95° (c 0.11, 50% aq. HOAc)  
 AAA: Asp, 2.0(2); Glu, 4.8(5); Ser, 1.6(2); His, 2.6(3); Gly,  
 1.1(1); Thr, 1.1(1); Arg, 7.3(7); Val, 0.8(1); Ile,  
 0.9(1); Leu, 8.3(8); Lys, 1.1(1); Ala, 1.8(2).

EXAMPLE VIIISynthesis of hPTHrp(1-34) analog gene

A synthetic gene coding for the hPTHrp(1-34) analog  
 Compound 4 (SEQ ID NO:9) was designed. The requisite  
 oligodeoxynucleotides were prepared with a DNA synthesizer  
 (Milligen/Bioscience) using the phosphoramidite process of  
 Sinha, et al., *Nucleic Acid Research* 12, 4539-4557 (1984).  
 After deprotection, the crude oligonucleotides were purified  
 by gel electrophoresis on preparative 15% polyacrylamide gels.  
 The oligonucleotides were located with UV, excised from the  
 gel, desalted over Waters c18 Sep-pak® cartridges, and  
 lyophilized.

Amplification via polymerase chain reaction (PCR) was  
 carried out on a Perkin-Elmer Cetus thermal cycler, with 25  
 cycles of: 94°C for 1 minute, 50°C for 2 minutes, and 72°C for  
 3 minutes, using reagents, including Taq polymerase, from the  
 "GeneAmp" DNA amplification kit (Perkin-Elmer Cetus).

Two overlapping oligonucleotides, an 88mer (2 µg), PTH3,

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(SEQ ID NO:31):

CCTCTAGATC TCCGCGGCGC TAGC ATG GCT GTT TCT GAA CAT CAG 45  
 Met Ala Val Ser Glu His Gln  
 1 5  
 5 CTG CTT CAT GAC AAA GGT AAA TCG ATT CAA GAT CTG AGA CGT C 88  
 Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg Arg  
 10 15 20

and an anti-sense 90mer (2 µg), PTH4 (SEQ ID NO:32):

10 CCTCGAAGCT TATGCATCAT TATCTAGA CAT AGT ATG CAG CTT TTC 46  
 Met Thr His Leu Lys Glu  
 30  
 15 AAG CAG TTT CTC CAG CAG CTC GCG ACG TCT CAG ATC TTG AAT 88  
 Leu Leu Lys Glu Leu Leu Glu Arg Arg Arg Leu Asp Gln Ile  
 25 20 15

CG 90,

were prepared as the template DNA sequence for the hPTHrp  
 (1-34) analog gene. Utilizing the two flanking primers,  
 PTHPCR1: CCTCTAGATC TCCGCGGCGC TAG (SEQ ID NO:33)  
 and PTHPCR2: CCTCGAAGCT TATGCATCAT TATC (SEQ ID NO:34), the  
 entire gene was amplified by PCR. The amplified DNA products  
 were purified by gel electrophoresis on 4% NuSieve® agarose  
 gel. The band containing the synthetic hPTHrp(1-34) analog  
 gene, approximately 150 bases in length, was excised from the  
 gel and approximately 200 ng of DNA isolated by Elu-Quik®  
 glass gel DNA extraction (Schleicher & Schuell, Keene, NH).

## EXAMPLE IX

Molecular Cloning of an hPTHrp(1-34)1 Analog Gene

To subclone the hPTHrp(1-34) analog gene of Example VI,  
 200 ng of the amplified DNA was isolated and cut by  
 restriction enzymes Hind III and Sac II. The DNA was ligated  
 to 2 µg TrpLE 18 Prot (Ile<sup>3</sup>,Pro<sup>5</sup>) plasmid previously cleaved  
 with Hind III and Sac II.

The resulting plasmid, TrpLE 18 hPTHrp(1-34)1, containing  
 one copy of the hPTHrp(1-34) analog gene was then transformed  
 into competent *E. coli* HB 101 cells (CLONTECH, Palo Alto, CA).  
 Transformants were subjected to PCR analysis to verify  
 insertion. Transformed cell colonies were selected and boiled  
 in 200 µL of water for 5 minutes; 2 µL were subjected to PCR



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with two primers flanking the insert. The PCR product was then analyzed on 1% agarose gel to confirm the presence of one copy of the hPTHrp(1-34) gene insert. TrpLE 18 hPTHrp(1-34)1 construct was then verified by DNA sequencing on an automated DNA sequencer (Applied Biosystems Model 373A, Foster City, CA) using the vendor's Dye Deoxy Terminator Sequencing kit.

#### EXAMPLE X

##### Construction of a Trp LE 18 vector containing multiple copies of the hPTHrp(1-34) analog gene

Unique Nhe I and Xba I restriction sites are located near the beginning and end of the hPTHrp(1-34) analog gene sequence. These two sites, which recognize different sequences, but produce identical single strand cohesive termini, allow the construction of multiple copy hPTHrp (1-34) genes within the Trp LE 18 vector.

In separate reactions, 5 µg of plasmid Trp LE 18 hPTHrp(1-34)1 containing a single copy of the gene was cleaved with Bam HI + Nhe I and Xba I + Bam HI. From each digest, about 300 ng of the fragment containing the hPTHrp(1-34) analog gene was isolated. These two fragments were mixed and ligated to form the Trp LE 18 hPTHrp(1-34)2 plasmid. This plasmid was used to transform competent *E. coli* HB 101 cells. Sizing of the transformed PCR products on 1% agarose gel was used to determine the presence of two copies of the hPTHrp(1-34) gene insert. TrpLE 18 hPTHrp(1-34)2 containing two copies of the gene was then confirmed by DNA sequencing. The correct fusion of the two hPTHrp(1-34) genes results in the elimination of Nhe I and Xba I sites at the junction. This makes the remaining Xba I and Nhe I sites flanking the tandem genes unique.

By repeating this process the final plasmid Trp LE 18 hPTHrp(1-34)4 containing four copies of the hPTHrp(1-34) gene was constructed. The sequence of Trp LE 18 hPTHrp(1-34)4 was found to be correct by DNA sequence analysis.

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**EXAMPLE XI****Expression and Purification of Trp LE 18 hPTHrp(1-34)4****Induction of the Trp LE 18 hPTHrp(1-34)4.**

A starter culture of 50 mL of LB culture media, J.H. Miller, "Experiments in Molecular Genetics," p.431 (1972), incorporated herein by reference, containing 50 $\mu$ g/mL ampicillin and 100 $\mu$ g/mL tryptophan, was inoculated with *E. coli* cells containing Trp LE 18 hPTHrp(1-34)4 plasmid, and grown overnight at 37°C with vigorous shaking to an  $A_{550}$  of about 6. Two liters of LB culture media for production were pre-warmed to 37°C and seeded with 20 mL of the starter culture to give an  $A_{550}$  of about 0.06. The culture was then grown with vigorous shaking to an  $A_{550}$  of between 0.6 and 0.8, whereupon 2 mL of a 10 mg/mL solution of indole acrylic acid (IAA) was added. Growth was continued with good aeration for about 16 hr to a final  $A_{550}$  of about 6 (typically, between 4 and 10). The cells were concentrated by centrifugation and resuspended in 500 mL of 50 mM Tris-HCl, pH 7.5, 0.1 mM EDTA buffer solution (Tris buffer).

The suspension was sonicated using a Heat Systems-Ultrasonics, Inc. model 220F sonicator (equipped with a 3/4" horn) operated at 50% of full capacity to avoid overheating.

To determine the extent of induction, the whole cells were analyzed by SDS-PAGE. The gene products derived from the TrpLE 18 hPTHrp(1-34)4 construct were seen as a major band of the predicted MW of approximately 17,000. This accounts for as much as 10% of the total cellular protein.

**Isolation of the Fusion Protein.**

The cell lysate was centrifuged for 15 min. at about 3600 x g to pellet the Trp LE 18 hPTHrp(1-34)4 fusion protein; the supernatant was discarded. The pellet was resuspended in 200 mL Tris buffer. (typically 40-80  $A_{550}$ /mL).

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**EXAMPLE XII****Processing of the Fusion Protein and Purification of homo-Serlactone hPTHrp(1-34) peptide**

5 Cleavage of the methionine residues flanking the hPTHrp(1-34) multimeric fusion protein with CNBr releases the desired homo-Serlactone hPTHrp(1-34) polypeptide, which was purified as described below.

**CNBr Treatment of Fusion Protein.**

10 The washed pellet of TrpLE 18 hPTHrp(1-34)<sub>4</sub> fusion protein was resuspended by gently stirring in 60 mL of 70% formic acid (about 20 mg/mL total protein; typically material from 1000 A<sub>550</sub> units of cells is dissolved in 3 mL). A few drops of octanol were added and N<sub>2</sub> bubbled through the solution  
15 for 20 minutes before adding 5.5 g CNBr. This reaction was allowed to proceed for 6 hours at 25°C before an equal volume of 50:50 MeOH:H<sub>2</sub>O was mixed with the sample and subsequently removed by rotary evaporation. After 2 to 4 repetitions of this process, the bulk of the formic acid and CNBr were  
20 essentially removed. The sample was then evaporated to dryness, redissolved in 200 mL water and lyophilized for storage.

**Purification of homo-Serlactone hPTHrp(1-34).**

25 The CNBr cleaved supernatant was dialyzed against 50 mM KH<sub>2</sub>PO<sub>4</sub> pH 6.5 for 24 hours with multiple changes. During dialysis, pH was maintained at 6.5. After dialysis, the precipitates were removed by high speed centrifugation. The supernatant was clarified through a Gelman 0.45μ filter device  
30 (Acrodisc 4184).

**Cation Exchange Chromatography.**

Initial purification was accomplished by cation exchange chromatography on a Bio-Gel TSK-SP-5PW HPLC column (21.5 x  
35 150mm). Chromatographic conditions for a flow rate of 8 mL/min and a yield of approximately 12 mg of highly purified homo-Serlactone hPTHrp(1-34) peptide were:

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1. Column equilibration in 50 mM  $\text{KH}_2\text{PO}_4$ , pH 6.5
2. Load 10 mL clarified supernatant (approximately 1.5 L culture broth or 2.4 g inclusion).
3. Wash column with 50 mM  $\text{KH}_2\text{PO}_4$  pH 6.5 containing 50 mM NaCl until baseline is stabilized.
4. Elute column with 50 mM  $\text{KH}_2\text{PO}_4$  pH 6.5 containing 90 mM NaCl. Collect fractions for about 45 minutes.
5. Analyze the 90 mM NaCl fractions for homo-Serlactone hPTHrp(1-34) content by C18 HPLC before pooling and storage.

#### Reverse Phase HPLC Chromatography.

A reverse phase Poros R/H 4.6 x 100 mm column (Perseptive Biosystems, Cambridge, MA) was used for the final purification step. The chromatographic conditions were as follows:

Mobile phase A: 0.1% trifluoroacetic acid (TFA)/water  
B: 0.1% trifluoroacetic acid (TFA)/ $\text{CH}_3\text{CN}$

	<u>TIME</u>	<u>FLOW</u>	<u>% B</u>
	0 min	4 ml/min	15
	5.0 min	4 ml/min	40
	5.2 min	4 ml/min	100
	6.8 min	4 ml/min	100
	7.0 min	4 ml/min	15

Retention time of the homo-Serlactone hPTHrp(1-34), Compound 4, was approximately 2.943 minutes. The purified peptide was approximately 98% pure as determined by mass spectroscopy.

#### EXAMPLE XIII

The compounds of this invention were evaluated for their effect on bone mass in ovariectomized rats, generally in accord with the procedures of Gunness-Hey and Hock, *Metab. Bone Dis.* 5:177 181 (1984).

Adult Sprague-Dawley female rats were acclimatized, weight grouped (n = 9, 10 or 12), and subjected to bilateral ovariectomy (OVX) or sham surgery. Dosing was initiated 17 days after surgery and continued for 20 days. Test compound was administered subcutaneously once a day in 2% rat

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serum/saline vehicle.

After 20 days of dosing, the rats were sacrificed and the right femurs excised. The femurs were cut in half and the distal half femurs (DHF) were further separated into trabecular bone (TB) and cortical bone (CB) by drilling out the trabeculae. Calcium was extracted and measured by Calcette calcium analyzer and expressed as mean bone Ca in mg/DHF/100 g body weight.

The two sample t-test was used to compare OVX and sham groups. One-way ANOVA was used to compare OVX groups, followed by Fisher's LSD multiple comparison to compare each treatment group to vehicle.

Ovariectomy induced substantial total bone loss, primarily from trabecular bone. Total bone calcium was 47 to 54% lower than for sham-operated controls.

bPTH(1-34) and hPTHrp(1-34) at 80 µg/kg/day provided statistically significant increases in total bone calcium for treated OVX rats, ranging from 53 to 95% and 18 to 40%, respectively; however, there was no significant increase in cortical bone calcium.

Compounds of this invention, dosed at 80 µg/kg/day, increased total bone calcium by from 66 to 138% and trabecular calcium by from 87 to 128%. Cortical bone calcium, trabecular thickness, and bone volume were also significantly increased over untreated OVX controls.

In this assay, the following compounds were tested:

<u>Compound Number</u>	<u>n (# of tests)</u>	<u>Trabecular Bone Calcium (% increase over OVX)</u>	<u>Total Bone Calcium (% increase over OVX)</u>
Compound 1 (SEQ ID NO:7)	6	101-128%	88-138%
Compound 2 (SEQ ID NO:6)	3	87-102%	66-114%
Compound 4 (SEQ ID NO:9)	3	-	-
88-114%			

In similar studies, ovariectomized rats were dosed for 5, 10 and 20 days, at 40 ug/kg/day, with the following results:

5	Compound Number	n (# of tests)	# of Days (d) of dosing	Total Bone Calcium (% increase over OVX)
	Compound 1 (SEQ ID NO:7)	3	20d	73-109%
	Compound 4 (SEQ ID NO:9)	5	20d	79-105%
10	Compound 4 (SEQ ID NO:9)	1	10d	79%
	Compound 49 (SEQ ID NO:63)	1	10d	93%
	Compound 4 (SEQ ID NO:9)	1	5d	55%
	Compound 42 (SEQ ID NO:56)	1	5d	60%

#### EXAMPLE XIV

15 Mature New Zealand White female rabbits (Hazelton Research Products, Inc., 3.6 kg, n=9-11/treatment group) were injected daily with vehicle or 0.15 mg/kg of prednisone for 13 months. Bone mineral density (BMD) was measured by dual

20 energy x-ray absorptiometry (Hologic Model QDR-1500W, Hologic, Inc., Waltham, MA) and biochemical markers of bone metabolism were followed. After 9 months, subgroups received in addition 0.05, 0.15 or 0.5 ug/kg/day sc of Compound 1 (Seq. ID No. 7). Two months later the doses were raised to 0.5, 3 and 10

25 ug/kg/day. Prednisone treatment resulted in rapid initial loss (2-4 months) followed by stable BMD for the remainder of the study. Final losses in BMD from baseline of  $12.4 \pm 1.3\%$ ,  $18.1 \pm 1.9\%$ , and  $11.8 \pm 2.5\%$  were observed in the lumbar spine A/P view, lateral view, and distal femur. Less decrease

30 ( $7.8 \pm 2.2\%$ ) was observed in the femoral diaphysis. The lower, early doses did not change these figures. After 30-60 days 10 ug/kg of Compound 1 in animals continuing on prednisone restored BMD at all sites to values not significantly

35 different from those seen in control rabbits treated with the vehicle alone for the 12 months. Serum osteocalcin fell 6.4 fold from a baseline of  $50 \pm 8$  ng/ml in prednisone treated rabbits, but was returned to normal by concomitant 10 ug/kg of Compound 1.

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## EXAMPLE XV

Nasal formulations of a peptide of this invention Compound 1 (SEQ ID NO: 7) were prepared and evaluated for efficacy in delivering the compound with high bioavailability.

Solutions containing 20 mg/ml of Compound 1, 0.2 mg/ml citric acid, 2.6 mg/ml sodium citrate, 0.2 mg/ml benzalkonium chloride, and varying amounts of sorbitol and different enhancers were prepared in sterile water and the pH adjusted to 6.0 with 1N NaOH.

The solutions were administered nasally to cynomolgous monkeys and the pharmacokinetics evaluated as described above.

The composition of the tested formulations and the results of in vivo testing are shown in Table 1 where it may be seen that the formulations of this invention provide bioavailabilities approaching 50% of that attainable by subcutaneous injection and thus may be suitable for clinical use in the treatment of osteoporosis.

Table 1. Nasal Formulations of PTHrp Analog (SEQ ID NO:7)

Formulation	A	B	C	D	E	F	G
Cmpd SEQIDNO7	20	20	20	20	20	20	20
Citric Acid	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium Citrate	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Benzalkonium Chloride	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sorbitol	30	30	12	---	35	40	35
Methylcellulose 1500	---	2.5	---	---	---	---	---
Propylene Glycol	---	---	10	50	---	---	---
Dimethyl- $\beta$ -cyclodextrin	---	---	---	---	50	---	---
Sodium Taurocholate	---	---	---	---	---	10	---
Sodium Glycocholate	---	---	---	---	---	---	10

Dose (mg)	N	Tmax (hours)	Cmax (ng/ml)	Ave. AUC (ng/ml hr)	Ave. Percent Bioavailability
B 4	3	0.3	2.7	1.7 $\pm$ 0.6	0.6 $\pm$ 0.2
C 4	6	0.3	2.8	2.7 $\pm$ 2.8	1.0 $\pm$ 1.0
D 4	3	0.3	4.0	3.3 $\pm$ 2.6	1.2 $\pm$ 1.0
D 1	6	0.4	0.5	1.1 $\pm$ 2.1	1.7 $\pm$ 3.0
E 4	8	0.3	40	36 $\pm$ 40	13 $\pm$ 15
F 4	6	0.6	119	128 $\pm$ 48	47 $\pm$ 18
G 4	6	0.3	157	133 $\pm$ 77	49 $\pm$ 29
Subcutaneous Injection:					
0.05	23	0.6	2.0	3.4 $\pm$ 2.8	100 $\pm$ 82

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Syntex (U.S.A.) Inc.
- (ii) TITLE OF INVENTION: PHARMACEUTICAL COMPOSITIONS FOR THE  
NASAL DELIVERY OF COMPOUNDS USEFUL FOR THE TREATMENT OF  
OSTEOPOROSIS
- (iii) NUMBER OF SEQUENCES: 86
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: Heller Ehrman White & McAuliffe
  - (B) STREET: 525 University Ave.
  - (C) CITY: Palo Alto
  - (D) STATE: CA
  - (E) COUNTRY: USA
  - (F) ZIP: 94301
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) AGENT INFORMATION:
  - (A) NAME: Chow, Y. Ping
  - (B) REGISTRATION NUMBER: 30,740
  - (C) REFERENCE/DOCKET NUMBER: 27610-04
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 415-324-7078
  - (B) TELEFAX: 415-324-0638

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: N-terminal



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## xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

	Ser	Val	Ser	Glu	Ile	Gln	Leu	Met	His	Asn	Leu	Gly	Lys	His	Leu
Asn	1				5					10					15
	Ser	Met	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val
His					20				25						30
	Asn	Phe													

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

	Ala	Val	Ser	Glu	Ile	Gln	Phe	Met	His	Asn	Leu	Gly	Lys	His	Leu
Ser	1				5					10					15
	Ser	Met	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val
His					20				25						30
	Asn	Phe													

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Ser	Val	Ser	Glu	Ile	Gln	Leu	Met	His	Asn	Leu	Gly	Lys	His	Leu	
1				5						10				15	
His	Ser	Leu	Glu	Arg	Val	Glu	Trp	Leu	Arg	Lys	Lys	Leu	Gln	Asp	Val
					20					25				30	

Asn Phe

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1						5								10	15
His	Asp	Leu	Arg	Arg	Arg	Phe	Phe	Leu	His	His	Leu	Ile	Ala	Glu	Ile
								20						25	30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

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(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
	1				5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Arg	Lys	Leu
					20				25					30	

Thr Ala

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
	1				5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
					20				25					30	

Thr Ala

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1				5				10					15	
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His					20				25					30	

Thr Ala

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 34  
 (D) OTHER INFORMATION: /note= "Xaa34 = homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1				5				10					15	
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His					20				25					30	

Thr Xaa

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 34

(D) OTHER INFORMATION: /note= "Xaa34 = homoserine  
lactone"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
	1				5				10					15	

His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
					20				25					30	

Thr Xaa

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
	1				5				10					15	

His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
					20				25					30	

	Thr	Ala	Gly	Arg	Arg
					35

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(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Lys Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
20 25 30

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Asp Leu Ala Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
 His 20 25 30

Thr Ala

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(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Gln Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
1 5 10 15

Asp Leu Arg Arg Ala Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
His 20 25 30

Thr Ala

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Gln Ala Val Ser Glu Ala Gln Leu Leu His Asp Leu Gly Lys Ser Ile  
1 5 10 15

Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
His 20 25 30

Ala Leu

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(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
Gln 1 5 10 15  
Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg Leu  
His 20 25 30  
Thr Ala

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
Gln 1 5 10 15  
Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg Leu  
His 20 25 30  
Thr Ala



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## (2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Arg	Gly	Arg	Ser	Ile
	1					5					10				15
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu	Lys	Arg	Leu
							20				25			30	
	Thr	Ala													

## (2) INFORMATION FOR SEQ ID NO:18:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 29
- (D) OTHER INFORMATION: /note= "Xaa29 = lysine-(OCCH<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>2</sub>OCH<sub>3</sub>) "

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Arg	Gly	Arg	Ser	Ile
	1					5					10				15

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Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Xaa Arg Leu  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO:19:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 29
- (D) OTHER INFORMATION: /note= "Xaa29 =  
 lysine-(OCCH<sub>2</sub>(OCH<sub>2</sub>CH<sub>2</sub>)<sub>110</sub>OCH<sub>3</sub>)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Arg Ser Ile  
 Gln 1 5 10 15

Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Xaa Arg Leu  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO:20:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1					5				10				15	

	Asp	Leu	Arg	Arg	Arg	Ala	Leu	Ala	Glu	Ala	Leu	Ala	Glu	Ala	Leu
His						20			25				30		

Thr Ala

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1					5				10				15	

	Asp	Leu	Arg	Arg	Arg	Ser	Leu	Leu	Ser	Ser	Leu	Leu	Ser	Ser	Leu
His						20			25				30		

Thr Ala

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1				5				10					15	

	Asp	Leu	Arg	Arg	Arg	Ala	Phe	Tyr	Asp	Lys	Val	Ala	Glu	Lys	Leu
His					20				25					30	

Thr Ala

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Xaa8 = norleucine"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 18
- (D) OTHER INFORMATION: /note= "Xaa18 = norleucine"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

	Ala	Val	Ser	Glu	Ile	Gln	Phe	Xaa	His	Asn	Leu	Gly	Lys	His	Leu
Ser	1				5				10					15	

	Ser	Xaa	Glu	Arg	Val	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His					20				25					30	

Asn Tyr

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## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Xaa8 = norleucine"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 18
- (D) OTHER INFORMATION: /note= "Xaa18 = norleucine"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

	Ala	Val	Ser	Glu	Ile	Gln	Phe	Xaa	His	Asn	Leu	Gly	Lys	His	Leu
Ser	1				5						10				15
	Ser	Xaa	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His					20				25					30	
	Asn	Tyr													

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

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Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1					5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
					20				25				30		
	Thr	Met	Ala												
			35												

## (2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: helical

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Xaa8 = glutamic acid or arginine"

## (ix) FEATURE:

- (A) NAME/KEY: Region
- (B) LOCATION: 1..10
- (D) OTHER INFORMATION: /note= "Sequence 26 is embedded at positions 22 to 31 of sequences 5, 6, 7, 8, 9, 10, 11, 12, 13, and 14."

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Glu	Leu	Leu	Glu	Lys	Leu	Leu	Xaa	Lys	Leu
1				5				10	

## (2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: helical

(ii) MOLECULE TYPE: peptide

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(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 8

(D) OTHER INFORMATION: /note= "Xaa8 = glutamic acid,  
lysine, or lysine-(OCCH2PEGX) "

(ix) FEATURE:

(A) NAME/KEY: Region

(B) LOCATION: 1..10

(D) OTHER INFORMATION: /note= "Sequence 27 is embedded at  
positions 22 to 31 of sequences 15, 16, 17, 18,  
and 19. "

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Glu	Leu	Leu	Glu	Arg	Leu	Leu	Xaa	Arg	Leu
1				5					10

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: helical

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(ix) FEATURE:

(A) NAME/KEY: Peptide

(B) LOCATION: 1..10

(D) OTHER INFORMATION: /note= "Sequence 28 is embedded at  
positions 22 to 31 of sequence 20."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ala	Leu	Ala	Glu	Ala	Leu	Ala	Glu	Ala	Leu
1				5					10

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

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- (D) TOPOLOGY: helical
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:
- (A) NAME/KEY: Peptide
  - (B) LOCATION: 1..10
  - (D) OTHER INFORMATION: /note= "Sequence 29 is embedded at positions 22 to 31 of sequence 21."
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
- |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Ser | Leu | Leu | Ser | Ser | Leu | Leu | Ser | Ser | Leu |
| 1   |     |     | 5   |     |     |     |     |     | 10  |

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 10 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: helical
- (ii) MOLECULE TYPE: peptide
- (iii) HYPOTHETICAL: NO
- (v) FRAGMENT TYPE: internal
- (ix) FEATURE:
- (A) NAME/KEY: Peptide
  - (B) LOCATION: 1..10
  - (D) OTHER INFORMATION: /note= "Sequence 30 is embedded at positions 22 to 31 of sequence 22."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ala	Phe	Tyr	Asp	Lys	Val	Ala	Glu	Lys	Leu
1				5					10

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 88 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA



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(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CCTCTAGATC TCCGCGGCGC TAGCATGGCT GTTCTGAAC ATCAGCTGCT TCATGACAAA  
60

GGTAAATCGA TTCAAGATCT GAGACGTC 88

(2) INFORMATION FOR SEQ ID NO:32:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 90 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

CCTCGAAGCT TATGCATCAT TATCTAGACA TAGTATGCAG CTTTCAAGC AGTTTCTCCA  
60  
GCAGCTCGCG ACGTCTCAGA TCTTGAATCG 90

(2) INFORMATION FOR SEQ ID NO:33:

- (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

CCTCTAGATC TCCGCGCGCT AGC 23

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## (2) INFORMATION FOR SEQ ID NO:34:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 24 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

CCTCGAAGCT TATGCATCAT TATC 24

## (2) INFORMATION FOR SEQ ID NO: 35:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

	Ala	Val	Ser	Glu	Ile	Gln	Phe	Leu	His	Asn	Leu	Gly	Lys	His	Leu
Ser	1				5						10				15
	Ser	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His					20					25					30
	Asn	Tyr													

## (2) INFORMATION FOR SEQ ID NO: 36:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 34

(D) OTHER INFORMATION: /note= "Xaa is homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

Ala	Val	Ser	Glu	Ile	Gln	Phe	Leu	His	Asn	Leu	Gly	Lys	His	Leu
Ser														
1				5						10				15

Ser	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His														
			20					25					30	

Asn Xaa

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 34

(D) OTHER INFORMATION: /note= "Xaa is homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

Ala	Val	Ser	Glu	Ile	Gln	Phe	Leu	His	Asn	Lys	Gly	Lys	His	Leu
Ser														
1				5						10				15

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His Ser Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
 20 25 30

Asn Xaa

## (2) INFORMATION FOR SEQ ID NO: 38:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

Gln Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 1 5 10 15

His Asp Leu Lys Leu Lys Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 39:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

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	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1					5				10					15
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu	Glu	Arg	Leu
His						20			25					30	
	Thr	Ala													

## (2) INFORMATION FOR SEQ ID NO: 40:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1					5				10					15
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu	Glu	Arg	Leu
His						20			25					30	
	Thr	Ala	Pro												
			35												

## (2) INFORMATION FOR SEQ ID NO: 41:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 37 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1					5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu	Glu	Arg	Leu
					20					25				30	
	Thr	Ala	Gly	Arg	Arg										
					35										

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 38

(D) OTHER INFORMATION: /note= "Xaa is homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
1					5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Arg	Leu	Leu	Glu	Arg	Leu
					20					25				30	
	Thr	Ala	Gly	Arg	Arg	Xaa									
					35										

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

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(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
Gln	1					5					10				15
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His						20				25					30

Thr Tyr

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Tyr	Ser	Ile
Gln	1					5					10				15
	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His						20				25					30

Thr Ala

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid

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(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Cys	Ser	Ile
Gln	1					5					10				15

	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His							20				25				30

Thr Ala

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 34 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 13

(D) OTHER INFORMATION: /note= "Xaa is  
Cys(CH-2CONH(CH-2)-2NH(biotinyl))"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Xaa	Ser	Ile
Gln	1					5					10				15

	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
His							20				25				30

Thr Ala



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## (2) INFORMATION FOR SEQ ID NO: 47:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13
- (D) OTHER INFORMATION: /note= "Xaa is  
Lys(7-dimethylamino-2-oxo-2H-1-benzopyran-4-acety  
l)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Xaa	Ser	Ile
	1				5					10				15	
His	Asp	Leu	Arg	Arg	Arg	Glu	Leu	Leu	Glu	Lys	Leu	Leu	Glu	Lys	Leu
			20						25					30	
	Thr	Ala													

## (2) INFORMATION FOR SEQ ID NO: 48:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

Gln	Ala	Val	Ser	Glu	His	Gln	Leu	Leu	His	Asp	Lys	Gly	Lys	Ser	Ile
	1					5					10			15	

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Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
His 20 25 30  
Thr Ala Gly  
35

(2) INFORMATION FOR SEQ ID NO: 49:

- ```
(i) SEQUENCE CHARACTERISTICS:
  (A) LENGTH: 33 amino acids
  (B) TYPE: amino acid
  (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 4
  (D) OTHER INFORMATION: /note= "Xaa4 is Glu(OCH-3)"

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 10
  (D) OTHER INFORMATION: /note= "Xaa10 is Asp(OCH-3)"

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 17
  (D) OTHER INFORMATION: /note= "Xaa17 is Asp(OCH-3)"

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 22
  (D) OTHER INFORMATION: /note= "Xaa22 is Glu(OCH3)"

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 25
  (D) OTHER INFORMATION: /note= "Xaa25 is Glu(OCH-3)"

(ix) FEATURE:
  (A) NAME/KEY: Modified-site
  (B) LOCATION: 29
  (D) OTHER INFORMATION: /note= "Xaa29 is Glu(OCH-3)"
```

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Xaa | His | Gln | Leu | Leu | His | Xaa | Lys | Gly | Lys | Ser | Ile |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
| His | Xaa | Leu | Arg | Arg | Arg | Xaa | Leu | Leu | Xaa | Lys | Leu | Leu | Xaa | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |
|     | Ala |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 4
- (D) OTHER INFORMATION: /note= "Xaa4 is Glu(OCH-3)"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 10
- (D) OTHER INFORMATION: /note= "Xaa10 is Asp(OCH-3)"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 17
- (D) OTHER INFORMATION: /note= "Xaa17 is Asp(OCH-3)"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 22
- (D) OTHER INFORMATION: /note= "Xaa22 is Glu(OCH-3)"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 25
- (D) OTHER INFORMATION: /note= "Xaa25 is Glu(OCH-3)"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 29

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(D) OTHER INFORMATION: /note= "Xaa29 is Glu(OCH-3)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Xaa | His | Gln | Leu | Leu | His | Xaa | Lys | Gly | Lys | Ser | Ile |
|     | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |
| His | Xaa | Leu | Arg | Arg | Arg | Xaa | Leu | Leu | Xaa | Lys | Leu | Leu | Xaa | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     | 25  |     |     |     | 30  |     |     |
|     | Ala |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:  
 (A) NAME/KEY: Modified-site  
 (B) LOCATION: 34  
 (D) OTHER INFORMATION: /note= "Xaa is homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
|     | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     | 25  |     |     |     | 30  |     |     |
|     | Thr | Xaa |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 35 amino acids

Thr Pro

[illegible]

Thr Arg Ser Ala Trp  
35

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## (2) INFORMATION FOR SEQ ID NO: 58:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Arg | Gly | Arg | Ser | Ile |
| 1   |     |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Arg | Leu | Leu | Glu | Arg | Leu |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
|     | Thr | Ala | Gly | Arg | Arg | Thr | Arg | Ser | Ala | Trp |     |     |     |     |     |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO: 59:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Arg | Gly | Arg | Ser | Ile |
| 1   |     |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Arg | Leu | Leu | Glu | Arg | Leu |
|     |     |     |     |     | 20  |     |     |     | 25  |     |     |     | 30  |     |     |



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Thr Arg Gly Arg Arg Thr Arg Ser Ala Trp  
           35                                  40

## (2) INFORMATION FOR SEQ ID NO: 60:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 42 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 13

(D) OTHER INFORMATION: /note= "Xaa13 is  
                           Lys(dihydrocinnamoyl)"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

Gln  Ala Val Ser Glu His Gln Leu Leu His Asp Arg Gly Xaa Ser Ile  
      1                          5                          10                          15

His  Asp Leu Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg Leu  
                           20                          25                          30

Thr Arg Gly Arg Arg Thr Arg Ser Ala Trp  
           35                                  40

## (2) INFORMATION FOR SEQ ID NO: 61:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site

(B) LOCATION: 8

(B) LOCATION: 8  
(D) OTHER INFORMATION: /note= "Leu8 is Norleucine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 18

(D) OTHER INFORMATION: /note= "Leu18 is Norleucine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Ala Val Ser Glu Ile Gln Phe Leu His Asn Leu Gly Lys His Leu

Ser 1 5 10 15

His Ser Leu Thr Arg Ser Ala Trp Leu Arg Lys Lys Leu Gln Asp Val  
20 25 30

Asn Tyr

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile

Gln 1 5 10 15

Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
His 20 25 30

Thr Met Ala  
35

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## (2) INFORMATION FOR SEQ ID NO: 63:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 33
- (D) OTHER INFORMATION: /note= "Xaa is Thr  
1,4-diaminobuteryl lactam"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
| Gln | 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |
|     | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
| His |     |     |     |     | 20  |     |     |     | 25  |     |     |     |     | 30  |     |

Xaa

## (2) INFORMATION FOR SEQ ID NO: 64:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
| Gln | 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     | 15  |     |

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Asp Leu Arg Arg Arg Phe Phe Leu Glu Lys Leu Leu Glu Lys Leu  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 65:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 Gln 1 5 10 15  
 Asp Leu Arg Arg Arg Glu Leu Leu His Lys Leu Leu Glu Lys Leu  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 66:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 Gln 1 5 10 15

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Asp Leu Arg Arg Arg Glu Leu Leu Glu His Leu Leu Glu Lys Leu  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 67:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 Gln 1 5 10 15  
 His Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Ile Ala Lys Leu  
 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 68:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 Gln 1 5 10 15

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Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Glu Ile  
 His 20 25 30

Thr Ala

## (2) INFORMATION FOR SEQ ID NO: 69:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 34
- (D) OTHER INFORMATION: /note= "Xaa is homoserine"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
 Gln 1 5 10 15

Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
 His 20 25 30

Thr Xaa

## (2) INFORMATION FOR SEQ ID NO: 70:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 34 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site

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(B) LOCATION: 34  
(D) OTHER INFORMATION: /note= "Xaa is homoserine"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
|     | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |     |
|     | Thr | Xaa |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 34 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: both

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
|     | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |     |
|     | Thr | Ala |     |     |     |     |     |     |     |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 37 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

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(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
Gln 1 5 10 15

Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
His 20 25 30

Thr Arg Ser Ala Trp  
35

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 36 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

```
(ix) FEATURE:
      (A) NAME/KEY: Modified-site
      (B) LOCATION: 36
      (D) OTHER INFORMATION: /note= "Xaa is Ala
          3-(2-naphthyl)-L-alanine"
```

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile  
Gln 1 5 10 15

His Asp Leu Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu  
20 25 30

Thr Arg Ser Xaa  
35



[illegible]

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## (2) INFORMATION FOR SEQ ID NO: 76:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
| 1   |     |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |
|     | Thr | Ala | Glu | Ile | Arg |     |     |     |     |     |     |     |     |     |     |
|     |     |     |     |     |     | 35  |     |     |     |     |     |     |     |     |     |

## (2) INFORMATION FOR SEQ ID NO: 77:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
| 1   |     |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     | 30  |

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Thr Ala Glu Ile  
35

## (2) INFORMATION FOR SEQ ID NO: 78:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 35 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Gln | Ala | Val | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
| His | Asp | Leu | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu |
|     |     |     |     |     | 20  |     |     |     | 25  |     |     |     |     | 30  |     |

Thr Ala Glu  
35

## (2) INFORMATION FOR SEQ ID NO: 79:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 34 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Leu8 is Norleucine"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 18
- (D) OTHER INFORMATION: /note= "Leu18 is Norleucine"

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## (ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) LOCATION: 34

(D) OTHER INFORMATION: /note= "Xaa is homoserine lactone"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | Ala | Val | Ser | Glu | Ile | Gln | Phe | Leu | His | Asn | Lys | Gly | Lys | His | Leu |
| Ser | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
|     | Ser | Leu | Glu | Arg | Val | Glu | Trp | Leu | Arg | Lys | Lys | Leu | Gln | Asp | Val |
| His |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

Asn Xaa

## (2) INFORMATION FOR SEQ ID NO: 80:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 32 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile | Gln | Asp |
| Leu | 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
|     | Arg | Arg | Arg | Glu | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu | His | Thr |
| Ala |     |     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |

## (2) INFORMATION FOR SEQ ID NO: 81:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Glu | Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile | Gln | Asp | Leu | Arg | Arg | Arg |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
|     | Leu | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu | His | Thr | Ala |     |     |     |
|     |     |     | 20  |     |     |     |     |     | 25  |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Leu | His | Asp | Lys | Gly | Lys | Ser | Ile | Gln | Asp | Leu | Arg | Arg | Arg | Glu |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |
|     | Leu | Glu | Lys | Leu | Leu | Glu | Lys | Leu | His | Thr | Ala |     |     |     |     |
|     |     |     | 20  |     |     |     |     |     | 25  |     |     |     |     |     |     |

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 41 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Leu | Ser | Glu | His | Gln | Leu | Leu | His | Asp | Arg | Gly | Arg | Ser | Ile | Gln | Asp |
| 1   |     |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |

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|     |                                                             |
|-----|-------------------------------------------------------------|
| His | Arg Arg Arg Glu Leu Leu Glu Arg Leu Leu Glu Arg Leu His Leu |
|     | 20 25 30                                                    |
|     | Arg Gly Arg Arg Thr Arg Ser Ala Trp                         |
|     | 35 40                                                       |

## (2) INFORMATION FOR SEQ ID NO: 84:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:

|     |                                                             |
|-----|-------------------------------------------------------------|
| Glu | Leu Leu His Asp Arg Gly Arg Ser Ile Gln Asp Leu Arg Arg Arg |
|     | 1 5 10 15                                                   |
| Arg | Leu Leu Glu Arg Leu Leu Glu Arg Leu His Ala Gly Arg Arg Thr |
|     | 20 25 30                                                    |
|     | Ser Ala Trp                                                 |
|     | 35                                                          |

## (2) INFORMATION FOR SEQ ID NO:85:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: helical

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(v) FRAGMENT TYPE: internal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site

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- (B) LOCATION: 1 and 4
- (D) OTHER INFORMATION: /note= "Xaa1 and Xaa4 = Glu,  
Glu(OCH3), His, or Phe"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Xaa2 = Leu or Phe"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 5
- (D) OTHER INFORMATION: /note= "Xaa5 = Lys or His"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 7 and 10
- (D) OTHER INFORMATION: /note= "Xaa7 and Xaa10 =  
Leu or Ile"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Xaa8 = Ala, Arg, or Glu"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 9
- (D) OTHER INFORMATION: /note= "Xaa9 = Lys or Glu"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Xaa | Leu | Xaa | Xaa | Leu | Xaa | Xaa | Xaa | Xaa |
| 1   |     |     | 5   |     |     |     |     |     | 10  |

## (2) INFORMATION FOR SEQ ID NO:86:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: helical

## (ii) MOLECULE TYPE: peptide

## (iii) HYPOTHETICAL: NO

## (v) FRAGMENT TYPE: internal

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1 and 4
- (D) OTHER INFORMATION: /note= "Xaa1 and Xaa4 = Glu,  
Glu(OCH3), His, or Phe"

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## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 2
- (D) OTHER INFORMATION: /note= "Xaa2 = Leu or Phe"

## (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 8
- (D) OTHER INFORMATION: /note= "Xaa8 = Glu, Lys or  
Lys(COCH2PEGX) "

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

|     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Xaa | Xaa | Leu | Xaa | Arg | Leu | Leu | Xaa | Arg | Leu |
| 1   |     |     |     | 5   |     |     |     |     | 10  |



We claim:

5 1. A pharmaceutical composition for the nasal delivery of compounds useful for treating osteoporosis, comprising an effective amount of a physiologically active truncated analog of PTH or PTHrp, or salt thereof, in which amino acid residues (22-31) form an amphipathic  $\alpha$ -helix, said residues (22-31) selected from (SEQ ID NOS: 85, 86, 26, 27, 28, 29, and 30); an  
10 absorption enhancer selected from the group consisting of dimethyl- $\beta$ -cyclodextrin and the bile acid surfactants; and water.

15 2. A composition of claim 1 wherein the bile acid surfactant is selected from the salts of glycocholic acid and taurocholic acid.

20 3. A composition of claim 2 wherein the bile acid surfactant is sodium taurocholate.

4. A composition of claim 2 wherein the bile acid surfactant is sodium glycocholate.

25 5. A composition of claim 1 wherein the concentration of PTH or PTHrP analog is from 1 mg/ml to about 100 mg/ml.

6. A composition of claim 1 wherein the concentration of absorption enhancer is from about 0.5 to about 50 mg/ml.

30 7. A composition of claim 1 wherein the PthrP analog is the compound of (SEQ ID NO:7).

8. A composition of claim 7 wherein the absorption enhancer is dimethyl- $\beta$ -cyclodextrin.

35 9. A composition of claim 7 wherein the absorption enhancer is sodium taurocholate.

10. A composition of claim 7 wherein the absorption enhancer is sodium glycocholate.

5 11. A pharmaceutical composition comprising from about 1 to about 100 mg/ml of a compound of (SEQ ID NO:7), from about 0.5 to about 50 mg/ml of an absorption enhancer selected from the group consisting of dimethyl- $\beta$ -cyclo-dextrin, sodium glycocholate, and sodium taurocholate, and water.